ADDIS ABABA UNIVERSITY

FACULTY OF VETERINARY MEDICINE

SEROPREVALENCE STUDY OF BOVINE BRUCELLOSIS AND ITS PUBLIC HEATH SIGNIFICANCE IN WESTERN TIGRAY, ETHIOPIA

BY

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A thesis submitted to the School of Graduate Studies of Addis Ababa University in partial fulfillment of the requirements for the Degree of Master of Veterinary Science in Tropical Veterinary Public Health

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ABBRIVATIONS

AAIPUDPS	Addis Ababa Intra and Peri-urban Dairy Production System
a.s.l.	above sea level
BgVV	Bandesinstitut fiir gesundheitlichen Verbraucherschutz and Veterinarmedizin
BOANRDT	Bureau of Agriculture and Natural Resource Development Tigray Regional State
CAR	Central Africa Republic
CHE	Central Highland of Ethiopia
CFT	Complement Fixation Test
CSF	Chaffa State Farm
FAO	Food ahd Agricultural Organization
EANRS	Eastern Amhara National Regional State
ELISA	Enzyme Linked Immunosorbent Assay
Ig	Immunoglobulin
LPS	Lipopolisaccharide
MRT	Milk Ring Test
NVI	National Veterinary Institute
NWE	North Western Ethiopia
OR	Odds Ratio
OIE	Office International Des Epizootics
RBPT	Rose Bengal Plate Test
rpm	revolution per minute
SDTH	Skin Delayed Type Hypersensitivity
SAT	Serum Agglutination Test
SEE	South Eastern Ethiopia
SRBC	Sheep Red Blood Cells
VCM	Veronal Calcium and Magnesium
WHO	World Health Organization
PAs	Peasant's Association
SPSS	Statistical Package for Social Science

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ABSTRACT

A cross sectional study was conducted to determine the seroprevalence and associations with potential risk factors of brucellosis in cattle and humans in western zone of Tigray Regional State between October 2007 and February 2008. A two-stage cluster sampling method was used in this study. A total of 1968 cattle were examined. Of these 1120 cattle were examined from semi-intensive production system and 848 cattle from extensive one. Human risk groups to brucellosis were purposively sampled. Sera sampling was carried out from all cattle above six months of age with no history of previous vaccination against brucellosis. The types of tests used to detect the presence of *Brucella* antibodies were Rose Bengal Plate Test and Complement Fixation Test. The overall individual animal prevalence was 4.9%. There were high significant differences (P=0.000) in seroprevalence to *Brucella* antibody between semi-intensive and extensive production systems. The seroprevalence to *Brucella* antibodies among nine towns in the semi-intensive and the three districts (Hmera, Tsegede and Welkait) at both individual and herd levels in the extensive production system were comparable. Seroreactor in both sexes of cattle were varied significantly. Females were more infected. Seroprevalence by categories of ages and herd sizes were significantly different. Higher risk to infection was found in *Barka* breeds than *Arado* in the semi-intensive production system but not in the extensive production system. History of abortion was found significantly (P=0.001) associated with brucellosis. Watering points and culling methods of management and husbandry related factors were significantly associated with seropositivity to brucellosis and brucellosis increased the calving intervals. In a total of 246 vulnerable groups of humans to brucellosis 1.2% overall seropositive to *Brucella* antibodies were obtained with all positive reactors being herdsmen. There was a high risk of acquiring the infection during removal of retained fetal membranes and in those who were both in contacts with animals and drank raw milk. A high prevalence of brucellosis in *Barka* breeds in the study area indicates that it might serve as source of infection for others in the region. Hence, before distributing this breed screening test for brucellosis is recommended. While in case of human brucellosis, since its presence is confirmed and the risk factors were identified, the medical personnel should give attention to its differential diagnosis with aims of offering specific therapies to true cases and creating awareness via education campaigns to human risk groups.

Key words: Breed, Cattle, Cross-sectional survey, Brucellosis, Human, Production system, Risk factors, Western Tigray

1. INTRODUCTION

Livestock agriculture plays a critical role for the majority of the Ethiopian population and remains the major source of draught power for ploughing and also serves an important source of food (Tegegne and Gebrewold, 1997). Livestock offers economic stability to reducing the risks associated with crop losses (John *et al.,* 2002). Even though Ethiopia, with its leading cattle population, significant number of other livestock in Africa, it is difficult to realize any gain in livestock production and productivity without first ensuring corresponding improvements in nutrition, management practices, animal health problems, which are the principal causes of poor performance leading to an ever increasing gap between the supply and the demand for livestock products (Taffese and Mekonnen, 1998).

Livestock diseases are major constraints for the socio-economic development of resource-poor farmers in the country. Extension in livestock management and disease prevention measures, including for zoonosis, is minimal and coverage of the total area by veterinary services is limited. Livestock diseases such as brucellosis are bottlenecks to the livestock production. Equally, many diseases including brucellosis are true zoonosis in that virtually all-human infections are acquired from animals (Coulibaly and Yameogo, 2000; Gebreyesus, 2001; Radostits *et al.,* 2000).

World wide, brucellosis remains an important disease in humans, domestic and wild animals (Tsolia *et al*., 2002). It is an infectious disease caused by the bacteria of the genus *Brucella.* These bacteria are primarily passed among animals and they cause disease in many different vertebrates. Various *Brucella* species affect sheep, goats, cattle, deer, pigs, dogs and several other animals. Brucellosis in animals is characterized by abortion in females, epididimitis and orchitis in males and infertility in both sexes (Walker, 1999; Omer *et al.,* 2000). Brucellosis is commonly transmitted to susceptible animals by direct contact with infected animals or with an environment that has been contaminated with discharges from infected animals. Aborted fetuses, fetal membranes or fluids, and other vaginal discharges after an infected animal has aborted or calved are all highly contaminated with infectious *Brucella* organisms. Animals may lick those materials or the genital area of other animals or ingest the disease causing organisms with contaminated feed or water. The disease may also spread when wild animals or animals from an infected herd mingled with *Brucella* susceptible free herds (Kubuafor *et al.,* 2000; Radostits *et al.,* 2000). A positive association between the numbers of animals kept in an area and the disease prevalence was reported attributing to increased contact among susceptible and infected animals (Menachem, 2002).

Brucellosis creates a serious economic problem for both intensive and extensive livestock production systems in tropics (John *et al.,* 2002). Its huge impact lies on losses of calves due to abortion in the 5^{th} to 8^{th} month of gestation, high incidence of retained fetal membrane and loss of a substantial milk production. It was reported that the loss could reach up to 40-50% in early abortion (Chukwu, 1987; Bishop *et al*., 1994). In intensive dairy production systems of the tropics, an incidence of infection up to 80% have been reported (Chukwu, 1987). Not enough with the livestock production losses, it is also a listed zoonotic disease. Brucellosis is considered by WHO, FAO and OIE as one of the most widespread zoonoses (Schelling *et al*., 2003). Human brucellosis is still the commonest zoonotic disease worldwide with over 500,000 new cases reported annually (Sascha *et al*., 2007). The mortality in human is low but the involvement of multiple organs may be debilitating for a long time (Sascha *et al*., 2007). It is usually an occupational disease occurring in animal health workers, farmers, stock inspectors, abattoir workers, laboratory personnel and butchers (Acha and Szyfers, 2001).

Brucellosis is perhaps the most widespread and economically important of the zoonotic diseases in tropical and sub-tropical regions. Distribution of the different species of *Bruclla* and their biovars varies across different geographic areas. *Bruclla abortus* is the most widespread species where as *Brucella melitensis* and *Brucella suis* are irregularly distributed (Quinn *et al*., 1999). Important factors that contribute to the prevalence and spread in livestock include farming system and practices, farm sanitation, livestock movement, mixing and trading of animals, sharing of grazing grounds and modern changes towards intensification (Kadohira *et al*., 1997; Omer *et al*., 2000; Kabagambe *et al*., 2001). Further complications arise through wild animal reservoirs that may also carry and transmit the disease (Godfroid, 2002), but the disease has been eradicated in most industrial countries, especially in Europe through intensive schemes of control and eradication system (Radostits *et al*., 2000).

In Ethiopia several investigators have established the endemicity of bovine brucellosis in different parts of the country (Bayleyegn, 1989; Abeje, 1994; Yilkal *et al.*, 1998; Tadesse, 2003; Mussie, 2005), and the available information on brucellosis clearly showed that the disease is endemic and wide spread with significant economic and public health importance in which the seroprevalence of bovine brucellosis was reported from zero to 22 % depending on the cattle management systems (Sintaro, 1994; Kelay, 2002). Moreover, brucellosis prevalence was found to be high in the more intensive management systems like dairy farms and ranches than in the extensively managed animals (Sintaro, 1994). On the other hand, no comparable data was available in western Tigray, a home to *Barka* cattle breeds.

Barka cattle, commonly known as *Begait*, is an indigenous zebu breed inhabiting South Sudan, Southern Eritrea and Northwest Ethiopia (Rege, 1999). The breed is mainly known for its milk production (which yields up to 12 L/day), meat and traction power. Like many tropical breeds, *Barka* has special adaptive traits, including disease tolerance, climatic tolerance, ability to use poor quality feed and to survive with irregular supplies of feed and water (Rege, 1999). Knowing all these merits of the breed, the regional government is providing these cattle to farmers in different districts to up-grade the genetic potentials of the low producing *Arado* breed cattle *(Bos indicus)* else where from West Tigray. Moreover, these relatively productive breeds are being distributed to various poverty-prone areas of the region and many new settlers amid to satisfying the demand of animal products (Meat and milk) within the shortest time possible.

Despite all the goals of local government (i.e., sustained food secured society by boosting livestock production), there is an evident gap on the practical implementation of this strategy when it comes to livestock distribution. Many farmers are skeptical on the perceived benefits of the breed. There are various complaints that there were problems of reproductive failure (one of the main symptoms of bovine brucellosis) in the said breed after distributing in various districts. In the existing situation, only around two third of the distributed cattle remained while the other one third were culled from individual farms due to the reasons mentioned above (Personal communication). For any sound implementation to take place, the epidemiology of production related diseases, such as, brucellosis should be clearly investigated. As brucellosis is zoonotic, capable of infecting humans by consumption of non-pasteurized milk and contact with infected animals, the epidemiological investigations should also constitute the extent to which humans are involved in the zoonoses.

Therefore the objectives of the study were:

- To determine the seroprevalence of brucellosis in selected cattle breeds
- To explore the potential risk factors associated with bovine brucellosis in these breeds and
- To investigate the presence of human seroreactors among vulnerable groups

2. LITERATURE REVIEW

2.1. Etiology

Brucellosis is an important zoonotic disease caused by infection with bacteria of the genus *Brucella* (Asbakk *et al.,* 1999). Bruce first isolated the causal organism from the liver of a patient died with undulated fever (Malta fever) (Seifert, 1996). The organism responsible for brucellosis is a small gram-negative, coccobacillus, non-spore forming, and non-motile facultative intracellular organism that has an external envelope demonstrated by electron microscope. *Brucella* are partially acid fast in that they are not decolorized by 0.5% acetic acid in modified Zeihl-Neelson and thus appear pink in blue background (Walker, 1999; Quinn *et al.*, 2002).

The genus *Brucella* is considered to consist of six species. These are *B. abortus*, *B. melitensis*, *B.suis*, *B. ovis*, *B. canis* and *B. neotomae* (OIE, 1996; Radostits *et al.*, 2000). Two new *Brucella* species, provisionally called *B. pinnipeddiae* and *B. cetaceae*, have been isolated from marine hosts within the past few years (Georgios *et al.*, 2005). Some of the *Brucella* species are further classified into biotypes (biovars) (Table 1).

Table 1: Nomenclatures and Characteristics of *Brucella* species.

Species	Biotype	Animal Hosts	Human Virulence*	Species Discrimination
B. melitensis	1–3	Goats, sheep, camels	++++	Fuchsin, positive; thionine, positive; safranin inhibition, negative; H_2S production, negative; urease, positive in 24 hr; CO_2 growth, negative.
B. abortus	1–6, 9	Cattle, camels, yaks, buffalo	++ to +++	Fuchsin, positive (except biotype 2); thionine, negative (biotypes 1, 2, and 4); safranin inhibition, negative; H_2S production, positive (except biotype 5); urease, positive in 24hr; CO_2 growth, positive (biotypes 1–4).
B. suis	1–5	Pigs	+	Fuchsin, negative (except biotype 3); thionine, positive; safranin inhibition, positive; H_2S production, positive (biotype1); urease, positive in 15 min; CO_2 growth, negative.
B. canis	-	Canines	+	Fuchsin, positive or negative; thionine, positive; safranin inhibition, negative; H_2S production, negative; urease, positive in 15 min; CO_2 growth, negative.
B. ovis	-	Sheep	–	Fuchsin, negative for some strains; safranin inhibition, negative; H_2S production, negative; urease, negative; CO_2 growth, positive.
B. neotomae	-	Rodents	–	Fuchsin, negative; safranin inhibition, negative; H_2S production, positive; urease, positive in 15 min; CO_2 growth, negative.
B. pinnipediae and *B. cetaceae*	-	Minke whales, dolphins, porpoises	+	Fuchsin, positive; thionine, positive; safranin inhibition, negative; H_2S production, negative; urease, positive; CO_2 growth, negative.

* Virulence is graded on a scale from no virulence (–) to the highest degree of virulence (++++).

Source: Coetzer and Tustin (2004)

2.2. Epidemiology

The epidemiology of brucellosis is complex. Important factors that contribute to the prevalence and spread in livestock include farming system and practices, farm sanitation, livestock movement, mixing and trading of animals, sharing of grazing grounds and modern changes towards intensification (Kadohira *et al.*, 1997; Omer *et al.,* 2000; Kabagambe *et al.*, 2001). Further complications arise through wild animal reservoirs that may also carry and transmit the disease (Godfroid, 2002).

2.2.1. Global perspective

Brucellosis occurs worldwide in domestic and game animals (Seifert, 1996). Bovine brucellosis has been eradicated from most industrialized countries, such as in Finland, Norway, Sweden, Denmark, Netherlands, Belgium, Switzerland, Germany, Australia, Hungary, the former Czechoslovakia, Rumania, Bulgaria and some others (Acha and Szyfers, 2001). In other parts of the world the rates of brucellosis caused by *B. abortus* vary greatly from one country to another and between regions within a country. The highest prevalence is seen in dairy cattle. Even in highly developed countries like USA and France have so far not been able to eradicate brucellosis completely. (Kubuafor *et al.,* 2000; Acha and Szyfers, 2001; John *et al.,* 2002).

2.2.2. Brucellosis in Africa

Brucellosis in cattle is prevalent in many countries of Africa (Kubuafor *et al.*, 2000). The reason for high prevalence is probably due to the fact that many countries have not yet started control or eradication schemes (Chukwu, 1985). Surveys on the prevalence of bovine brucellosis have been carried out in some African countries (Table 2). Brucellosis status in humans in Africa is also considerable (Table 3).

Table 2: Prevalence of bovine brucellosis in some African countries

Country	n*	Prevalence (%)	Test used	References
Uganda	315	15.8	RBPT	Bernard *et al.* (2005)
Tanzania	417	12.2	SAT	Swai *et al.* (2005)
Angola	1175	7.9	RBPT	Parodi *et al.* (2004)
CAR	2032	3.3	RBPT	Nakoune *et al.* (2004)
Burkina Faso	290	13.2	RBPT	Traore *et al.* (2004)
Benin	710	15.2	ELISA	Koutinhouin *et al.* (2003)
Chad	1637	7	ELISA	Schelling *et al*. (2003)
Sudan	1038	5	RBPT, CFT	El-Ansary *et al.* (2001)
Eritrea	1356	8.2	RBPT, CFT	Omer *et al*. (2000)
Ghana	183	6.6	SAT, CFT	Kubuafor *et al. (2000)*
Nigeria	398	4.3	RBPT, SAT	Olayinka *et al.* (2000)
Zambia	291	32.7	RBPT, SAT	Ahmadu *et al.* (1999)
Nigeria	4497	22.3	ELISA	Garba and Obi (1998)
Djibouti	499	4	CFT	Chantal *et al.* (1996)
Tanzania	13078	10.8	SAT	Jiwa *et al*. (1996)
Rwanda	654	34.9	CFT	Akakpo *et al.* (1987)
Guinea	1861	6.9	RBPT, CFT	Chukwu (1985)
Egypt	50	100	SAT, MRT	Chukwu (1985)
Nigeria	133	79.7	SAT	Chukwu (1985)
Togo	1056	41	RBPT, CFT	Chukwu (1985)

n* =Total number of animals tested

Table 3: Human seroreactors to *Brucella* antibody in Africa

Country	Number tested	Prevalence (%)	Serology	References
Chad	860	3.8	ELISA	Schelling *et al.* (2003)
Sudan	187	1.0	RBPT, CFT	El-Ansary *et al.* (2001)
Egypt	330	0.09	SAT	Abou-Eisha (2000)
Eritrea	130	7.1	CFT	Omer *et al*. (2000)
Djibouti	108	6.5	CFT	Chantal *et al*. (1996)
Somalia	353	0.6	SAT	Hassein *et al.* (1987)
Nigeria	13999	7.6-29.8	SAT	Chukwu (1985)
Tanzania	540	22.6	SAT	Chukwu (1985)
Uganda	3164	6.4	SAT	Chukwu (1985)

2.2.3. Bovine Brucellosis: An Ethiopian Perspective

Studies on the prevalence of bovine brucellosis have been carried out in many parts of Ethiopia. These studies were conducted in local and cross breed animals. In these studies seroprevalence of brucellosis in cattle ranging from 0.2% up to 22% were recorded (Table 4). Human brucellosis in Ethiopia has been also indicated to exist although enough prevalence estimations are lacking (Table 5).

Table 4: Summary of seroprevalence of bovine brucellosis in Ethiopia

Locations	Breed	n*	Prevalence (%)	Tests	Sources
Tigray	Cross	816	3.19	RBPT, CFT	Gebretsadik (2005)
Bahirdar	Cross	1135	0.26	RBPT, CFT	Mussie (2005)
Sidamo Zone	Cross	811	2.5	RBPT, CFT	Kassahun (2004)
	Local	1627	1.7		
Jimma zone	Cross	805	0.8	RBPT, CFT	Tadele (2004)
	Local	1305	0.2		
Tigray	Cross	300	0.2	RBPT, CFT	Aberha (2003)
	Local	100	0.69		
Gonder Zone	Cross	1944	4.63	RBPT, CFT	Tadesse (2003)
NWE	Cross	4243	8.3	RBPT, CFT	Mekonnen (2002)
SEE	Cross	4243	4.9	RBPT, CFT	Abay *et al*. (2000)
EANRS	Local	3644	1.8	RBPT, CFT	Kebede (1999)
AAIPUDPS	Cross	1114	8.1	RBPT, CFT	Yilkal *et al.* (1998)
Bahirdar	Cross	1855	16.55	RBPT, SAT	Abeje (1994)
CSF	Cross	182	22	RBPT, SAT	Sintaro (1994)
Arsi	Cross	2178	8.86	RBPT, CFT	Bayleyegn (1989)
Ghibe, Gobe	Local	1606	4.2	RBPT, CFT	Tekleye *et al.* (1989)
CHE	Cross	3577	2.1	SAT, CFT	Assegid (1987)

n* =Total number of animals tested

Table 5: Human seroreactors to *Brucella* antibody in Ethiopia

Locations	Total tested	Number of Positives	Serology	References
Bahirdar	238	9	RBPT, CFT	Mussie (2005)
Sidama	38	2	RBPT, CFT	Kassahun (2004)
Jimma	126	3	RBPT, CFT	Tadele (2004)
NWE	49	12	RBPT	Gebreyesus (2001)
Abernosa	8	1	RBPT, CFT	Taye (1991)

2.3. Source of infection and determinant factors affecting transmission of brucellosis

2.3.1. In animals

Brucelosis is commonly transmitted to susceptible animals by ingestion of contaminated feed and water, contact with aborted fetuses, fetal membranes and uterine discharges, exposure through the conjunctival and genital mucosa, skin and respiratory routes occur while venereal transmissions of *B. suis* in swine, *B. ovis* in sheep and *B. canis* in dogs are common (Walker, 1999; Acha and Szyfers, 2001).

Ingestion of milk from infected cattle is another source for infection of calves. The disease may also be spread when wild animals or animals from an affected herd mingle with brucellosis free herds. Infection by aerosols and airborne infection has also been demonstrated (Acha and Szyfers, 2001; Muma *et al*., 2007). The use of infected bulls for artificial insemination poses an important risk and spreads the infection to many herds (Chukwu, 1987). Environment, host and agent factors are main determinant factors affecting transmission of brucellosis.

Environment: The survival of the organism in the environment plays a great role in the epidemiology of the disease. Temperature, humidity and pH influence the organism's ability to survive in the environment. *Brucella* are sensitive to direct sunlight, disinfectants and pasteurization. The organisms rarely survive in sour milk, sour cream and butter, or fermented cheese. It can survive in tap water for several months at 4-8 ^{0}C, 2.5 years at 0 ^{0}C and several years in frozen tissues or medium. *Brucella* can also survive up to 60 days in damp soil and up to 144 days at 20 ^{0}C and 4% relative humidity (Radostits *et al.,* 2000; John *et al*., 2002).

Management by which animals are reared also contributes a role to the transmission of the agent. Once infected, the time required to become free from brucellosis is increased by large herd size, active abortions and loose housing (Mohamed, 2002). Calving practices play a major role in the spread of brucellosis. Separate calving pens minimize exposure of infected animals. There is a positive association between population density (number of animals per land area) and disease prevalence, which is attributed to increased contact between susceptible and infected animals.

Management practices directed at eliminating infected males and minimizing exposure to aborted tissue greatly reduce the incidence of the disease. Both venereal transmission and exposure to aborted fetuses and fetal membranes are crucial for maintaining infections in a herd. Introduction of infected animals can lead to rapid spread of infection within the flock (Walker, 1999; Menachem, 2002).

Host factor: Susceptibility to infection depends on age, sex, breed and pregnancy status of the animal. Younger animals tend to be more resistant to infection and frequently clear infections than sexually matured. Mature animals are much more susceptible to infection, regardless of sex. In female animals pregnancy has positive contribution to the degree of susceptibility than their age (Walker, 1999; John *et al*., 2002). Bulls are relatively resistant than sexually mature heifers and less resistant than sexually immature heifers. In contrast to bull, boars are more likely to be a source for introducing *Brucella* into a swineherd (Walker, 1999; Acha and Szyfers, 2001). Moreover all breeds of cattle appear to be comparable in susceptibility to brucellosis and apparently no specific breed resistance to brucellosis is known. Rather there are varying degrees of individual resistance to infection, which are dependent upon gestation, exposure dose, age, vaccination and unknown host resistance factors (Nicoletti, 1984; Coetzer and Tustin, 2004).

Agent factors: *Brucella* are facultative intracellular bacteria, which are capable of multiplication and survival with in host phagocytes (WHO, 1997). The organisms are able to survive within host leukocytes and may utilize both neutrophils and macrophages for protection from humoral and cellular bactericidal mechanism during the period of haematogenous spread. The inability of the leukcytes to effectively kill virulent *B. abortus* at the primary site of infection is a key factor in the dissemination to regional lymphnodes and other sites such as recticuloendothelial system and organs such as the uterus and udder (Radostits *et al*., 2000; Georgios *et al*., 2005).

2.3.2. In humans

Brucellosis in humans is acquired from infected animals through direct contact or indirectly by ingestion of animal products and by inhalation of airborne agents (Acha and Szyfers, 2001). The relative importance of the etiologic agent's mode of transmission and pathway of penetration varies with the epidemiological area, the animal reservoirs and occupational groups at risk.

Human brucellosis caused by *B. melitensis* is the most sever one followed by *B. suis, B. abortus* and *B. canis* and recently new types reported in marine mammals eventually pathogenic to man. *B. ovis* and *B. neotomae* are not pathogenic to humans (Coulibaly and Yameogo, 2000; Mohamed, 2002). Consumption of unpasteurized dairy products especially raw milk, soft cheese, butter and ice cream is the most common means of transmission. Hard cheese, yogurt, and sour milk are less hazardous, since both propionic and lactic fermentation takes place (John *et al*., 2002). Human infections also occurred during laboratory manipulation of culture for vaccine or antigen production or diagnosis and infection due to accidental self-inoculation during the administration of live vaccine (Kubuafor *et al.,* 2000; Memish, 2001). Though bacterial load in animal muscle tissue is low, consumption of undercooked traditional delicacies such as liver and spleen has been implicated in human infection. It is also possible for raw vegetables and water contaminated with the excreta of infected animals to serve as sources of infection (Seifert, 1996; Georgis *et al*., 2005). Transmission by contact predominates in areas where bovine and porcine brucellosis are enzootic. Man may be infected by the handling of the tissues of diseased animals or by close contact with other infectious materials, presumably the *Brucella* enter through minute abrasion in the skin or possibly through the intact skin and transconjunctival and airborne transmission has been proven. Humans are accidental and almost always dead end hosts of *Brucella* infections; man to man transmission is also a rare possibility and no human to lower animal transmission has been reported (Nicoletti, 1984; Tsolia *et al.,* 2002).

2.4. Pathogenesis and clinical features

The initiation of *Brucella* infection depends on exposure dose, virulence of the *Brucella* species and natural resistance of the animal to the organism (Hirsh and Zee, 1999; Radostits *et al*., 2000). Resistance to infection is based on the host's ability to prevent the establishment of infection by the destruction of the invading organism. Invading *Brucella* usually localize in the lymph nodes, draining the invasion site, resulting in hyperplasia of lymphoid and reticlo-endothelial tissue and the infiltration of inflammatory cells. Survival of the first line of defense by the bacteria results in local infection and the escape of *Brucella* from the lymph nodes into the blood (Hirsh and Zee, 1999; Coetzer and Tustin, 2004). During the bacteraemic phase, which may last 2-8 weeks, bones, joints, eyes and brain can be infected, but the bacteria are most frequently isolated from supramammary lymph nodes, milk, iliac lymph nodes, spleen and uterus. In bulls, the predilection sites for infection are the reproductive organs and the associated lymph nodes. During the acute phase of infection, the semen contains large number of *Brucella* but the infection becomes more chronic, the number of *Brucella* excreted decreases and excretion may cease altogether. However, it may also continue to be excreted for years or just become intermittent. (Hirsh and Zee, 1999; Radostits *et al.*, 2000).

After the *Brucella* organisms spread through the hematogenous route in females it also reaches the placenta and then to the fetus. The preferential localization to the reproductive tract of the pregnant animals is due to the presence of unknown factors in the gravid uterus. These are collectively referred to as allantoic fluid factors that would stimulate the growth of *Brucella*. Erythritol, a four-carbon alcohol, is considered to be one of these factors (Walker, 1999), which are elevated in the placenta and fetal fluid from about the fifth month of gestation (Bishop *et al.*, 1994). An initial localization with in erythrophagocytic trophoblasts of the placentome, adjacent chorioallantoic membrane results in rupture of the cells and ulceration of the membrane. The damage to placental tissue together with foetal infection and foetal stress inducing maternal hormonal changes may cause abortion (Seifert, 1996).

Abortion and expulsion of the fetus was thought to be the results of placentitis caused by *Brucella*. Proliferation of *Brucella* in the uterus induces necrosis and destruction of the fetal and maternal placental membranes resulting in death and then expulsion of the fetus. The pathologic changes in the caruncules and cotyledons prevent normal separation and expulsion of the placenta. Although placentitis impairs the normal function of the placenta, *Brucella* endotoxins may also play a role in inducing abortion (Radostits *et al.*, 2000).

The primary clinical manifestations of brucellosis are related to the reproductive tract. The most obvious signs in pregnant animals are abortion or birth of weak calves, lowering of fertility with poor conception rates, retained fetal membrane, reduced milk yield. Abortion usually occurs between the fifth and seventh months of pregnancy. Seminal vesicles, ampullae, testicles and epydidimis may be infected in bulls (Nicoletti, 1984; Seifert, 1996). Chronic infection with *B. abortus* in cattle can result in hygroma (Walker, 1999).

In humans the onsets of clinical signs occur within 2-3 weeks of exposure to infection. Clinical signs include recurrent fever, chills with night sweats, fatigue, muscle joint pain, backache, depression and insomnia are common. In chronic form it may resulted with serious complications in which the musculo-skeletal, cardiovascular and central nervous systems are affected (Morata *et al*., 2003; Coetzer and Tustin, 2004).

2.5. Immunity

2.5.1. Humoral immune response

The antibody response to *B. abortus* in cattle consists of an early IgM isotypes response, the timing of which depends on the route of exposure, the dose of bacteria and the health status of the animal. Naturally, infected animals and those vaccinated, as adults with strain 19 remain positive to the serum and other agglutination tests for long periods. The serum of infected cattle contains high levels of IgG1, IgG2, IgM and IgA isotypes of antibody (Radostits *et al*., 2000). Similar isotyps different at relative concentrations occur in milk, although most of the IgA is present in secretory form. The first isotype produced after an initial heavy infection or strain 19 immunization is IgM and is soon followed by IgG antibody. IgG1 is the most abundant in serum and exceeds the concentration of IgG2. The magnitude and duration of the antibody response following immunization is directly related to the age at immunization and the number of organisms administered. Following immunization with a standard dose of strain 19 during calf hood, IgG antibody concentrations usually decline to diagnostically insignificant levels over 3-6 months. Residual antibody if present, is usually predominantly of the IgM class. Following exposure to virulent *B. abortus,* antibody may appear in 4-10 weeks or longer, depending on the size and route of entry of the inoculums and the stage of pregnancy of the animal. Antibodies of IgG, and IgM isotypes can all react in the tube agglutination test (WHO, 1997; OIE, 2000c).

2.5.2. Cellular immune response

Brucella species are facultative intracellular pathogens. They are readily phagocytised by macrophages and polymorphonuclear leukocytes and in the case of virulent strains, are capable of surviving within these cells, and phagocytosis is promoted by antibody. However, since virulent *Brucella* can survive within normal macrophages for long periods, recovery from infection is likely to be dependent upon the acquisition of increased bactericidal activity by phagocytic cells. Macrophage activation occurs when T-lymphocytes of the appropriate subset are stimulated to release lymphokines (interleukins). (WHO, 1997; Coetzer and Tustin, 2004; Georgios *et al.*, 2005) The release of these activating factors is dependent upon recognition of the appropriate antigen by the T-lymphocyte and is subject to regulation through the major histocompatabilty complex. Live organisms capable of establishing persistent intracellular infection and certain types of antigens, with or with out adjuvant, are the most effective inducers of cell-mediated immunity. The role of cytotoxic cells, including cytotoxic T-lymphocyts, natural killer cells (NK) and killer (K) cells, in the cell-mediated immune response to *Brucella* has not been elucidated. Further studies are needed to determine the basic processes underlying the developments of protective immunity to *Brucella* in the natural host species (WHO, 1997; Coetzer and Tustin, 2004).

2.6. Signifcance of brucellosis

2.6.1. Economic importance

Brucellosis causes heavy economic losses in livestock producers. The economic losses stem from abortion, reduced milk production, losses of calves due to abortion, culling and condemnation of valuable cows, endangering animal export trade of a nation particularly when other countries purchase only *Brucella* free animals, losses of man-hours and medical costs and government costs incurred for research and eradication programs (Chukwu, 1985; Abay *et al.*, 2000; Georgios *et al.*, 2005).

The common sequel of infertility increases the period between lactations in infected herds, and the average intercalving period may be prolonged by several months (Radostits *et al.,* 2000). In 1934 the estimated annual economic loss due to brucellosis in USA was US $ 100 million. Similarly, in Sweden prior to the eradication of brucellosis in 1957, the annual economic loss was estimated well above US $ 8.8 million (Schwable, 1984). Official estimates put annual economic losses due to bovine brucellosis in Latin America at approximately US $ 600 million (Acha and Szyfers, 2001). The annual economic loss from bovine brucellosis of 150 million French Francs in Cotedivore, 233.88 million US $ in Nigeria and 33.4 million US $ in Kenya, Tanzania and Uganda have been recorded (Chukwu, 1987). This signifies infection with brucllosis poses huge economic wastage in the livestock sector.

2.6.2. Human zoonoses risk

Human brucellosis is widely distributed all over the world, with high endemicity in the Miditerranean, Middle East, Latin America and parts of Asia (Corbel, 1997; Kalaajieh, 2000). It is considered by the Food and Agriculture Organization (FAO), the World Health Organization (WHO) and the Office International des Epizootis (OIE) as one of the most wide spread zonooses in the world (Schelling *et al.*, 2003). But the true incidence of human brucellosis is unknown (Garrido-Abellan *et al.*, 2001). More than 500,000 new cases are reported each year though the figure was subjected to underestimation (WHO, 1997). In Saudi Arabia 7,893 human cases of brucellosis were recorded, 74 per 100,000 inhabitants (Acha and Szyfers, 2001; Memish, 2001). In Iran 71,051 cases, 13 per 100,000 were recorded in 1988, and it is estimated that 80,000 cases have occurred each year since 1989 and in Turkey 5003 cases, 9 per 100,000 were recorded in 1990 (Acha and Szyfers, 2001). This is due to the consumption of fresh unpastuerized milk from sheep, goats and camels is a traditional practice, and in Saudi Arabia it is considered the main source of brucellosis in persons, offering fresh milk to visitors is a sign of hospitality in the culture and boiling is believed to remove the goodness from the milk (Memish, 2001).

The significance of brucellosis as a zonoosis has ever increased in recent times, as a result of expansion of international commerce in animals and animal products, increase urbanization with growing demand-supply of animals and animal products in closest proximity to people, increasing tourism (consumption local animal products), and new methods of cattle production involving higher animal concentration, having with nomadic animal husbandry, new ways of land use (irrigation) (Weidman, 1991; Coulibaly and Yameogo, 2000; Menachem, 2002). Brucellosis is most common in rural areas and it also occurs in urban settings where animals are kept in compounds around houses and among meat packers and veterinarians (Smits *et al.*, 1999). Human brucellosis is a disease with non-pathognomonic signs and characterized by acute illness with undulant fever, which may progress to a more chronic form and can also produce a serious complication affecting the musculoskeletal, cardiovascular and central nervous system (OIE, 1997; Georgios *et al*., 2005).

2.7. Diagnosis

Diagnosis of brucellosis is the corner stone for any control and eradication program. Especially in humans due to its heterogeneous and poorly specific clinical symptoms the diagnosis of brucellosis always requires laboratory conformation (Morata *et al*., 2003). It is made possible by direct demonstration of the causal organism using staining, immunofluorescent antibody, culture, animal inoculation and Polymerase Chain Reaction (PCR) and indirectly by demonstration of antibodies using serological techniques (Corbel, 1997; Walker, 1999; Quinn *et al*., 2002).

2.7.1. Microscopic examination and cultural methods

Specimens of fetal stomach, lung, liver, placental cotyledon, vaginal discharges, are stained with Gram stain and modified Ziehl Neelsen stains. *Brucella* appear as small red-colored coccobacilli in clumps. Blood or bone marrow samples can be taken and cultured in 5-10% blood agar is used. To check up bacterial and fungal contamination *Brucella* selective medias are often used. The selective medias are nutritive media blood agar based with 5% seronegative equine or bovine serum. On primary isolation it usually requires the addition of 5-10% carbon dioxide and takes 3-5 days incubation at 37 ^{0}C for visible colonies to appear (Quinn *et al*., 2002). The organism is catalase and oxidase positive, (*Brucella ovis* and *Brucella neotomae* are oxidase negative), reduce nitrate to nitrite (except *Brucella ovis*), *Brucella* does not cause haemolysis on blood agar, does not produce acid on agar containing glucose, and does not ferment lactose (Walker, 1999; Quinn *et al*., 2002).

2.7.2. Animal inoculation

Guinea pigs are the most sensitive laboratory animals, two guinea pigs are inoculated intramuscular 0.5-1.0ml of suspected tissue homogenate and are sacrificed at three and six weeks post inoculation and serum is taken along with spleen and other abnormal tissues for serology and bacteriological examination, respectively (Walker, 1999; Quinn *et al*., 2002).

2.7.3. Serological diagnosis

Body fluids such as serum, uterine discharge, vaginal mucus, milk, or semen plasma from suspected cattle may contain different quantities of antibodies of the IgM, IgG1, IgG2, and IgA types directed against *Brucella* (Georgios *et al*., 2005). Because infected cattle may or may not produce all antibody types in detectable quantities several tests are used to detect brucellosis. The commonly used tests are the milk ring test (MRT), serum agglutination test (SAT), complement fixation test (CFT), Rose Bengal (RB) plate test, anti-globulin (Coombs) test, 2-mercaptoethanol, rivanol and the enzyme-linked immunosorbent assay (ELISA). The use of several tests to reliably detect brucellosis suggests shortcomings in each of the tests (Klaus, 2002).

Milk ring test (MRT): The milk ring test (MRT) is cheap, easy, simple and quick to perform. It detects lacteal anti- *Brucella* IgM and IgA bound to milk fat globules. However, it tests false positive when milk that contains colostrum, milk at the end of the lactation period, milk from cows suffering from a hormonal disorder or milk from cows with mastitis are tested (Nielsen *et al.,* 2001). Milk that contains low concentrations of lacteal IgM and IgA or lackes the fat-clustering factors tests false negative (Klaus, 2002). Because lacteal antibodies rapidly decline after abortion or parturition, the reliability of the MRT, using 1 ml milk, to detect *Brucella* antibodies in individual cattle or in tank milk is strongly reduced (OIE, 2000c). Although the MRT performed with 8 ml milk it improved the detection of brucellosis in tank milk, it may test false positive when traces of colostrum are present in tank milk (Georgios *et al*., 2005).

Serum agglutination test (SAT): The serum agglutination test (SAT), which historically has been the principal serological test used to detect brucellosis, measures agglutinating antibodies of the IgM, IgG1, IgG2, and IgA types (Nielsen et al., 2001). The SAT is relatively simple and easy to perform but it requires basic laboratory equipment. It can be used to detect acute infections, as antibodies of the IgM type usually appear first after infection and are more reactive in the SAT than antibodies of the IgG1 and IgG2 types; however, because the SAT may yield both false negative or false positive results, it effectively detects brucellosis only on a herd basis (Corbel *et al.*, 1984).

Complement fixation test (CFT): The complement fixation test (CFT) detects specific antibodies of the IgM and IgG1 type that fix complement (Georgios *et al.*, 2005). The CFT is highly specific but it is laborious and requires highly trained personnel as well as suitable laboratory facilities. This makes the CFT less suitable for use in developing countries. Although its specificity is very important for the control and eradication of brucellosis it may test false negative when antibodies of the IgG2 type hinder complement fixation. The CFT measures more antibodies of the IgG1 type than antibodies of the IgM type, as the latter are partially destroyed during inactivation. Since antibodies of the IgG1 type usually appear after antibodies of the IgM type control and surveillance for brucellosis is best done with SAT and CFT (MacMillan, 1990; Nielsen *et al.*, 2001).

Rose Bengal plate test (RBPT): The Rose Bengal plate test is a spot agglutination technique. Because the test does not need special laboratory facilities and it is simple and easy to perform, which is used to screen sera for *Brucella* antibodies. The test detects specific antibodies of the IgM and IgG types and is more effective in detecting antibodies of the IgG1 type than IgM and IgG2 types (Nielsen *et al.,* 2001). The test may yield positive results in non-infected cattle that give negative results with the CFT (OIE, 2000c). Although the low pH (3.6) of the antigen enhances the specificity of the test and the temperature of the antigen and the ambient temperature at which the reaction takes place may influence the sensitivity and specificity of the RBPT test (MacMillan, 1990).

Anti-globulin (Coombs) test: The anti-globulin (Coombs) test detects (incomplete Brucella) antibodies of the IgG2 type and is used to confirm SAT results (OIE, 2000c). The Coombs test, although laborious, is particularly important when the SAT is positive and CFT results are negative or inconclusive (Kerkhofs et al., 1990). However, Coombs test results are indicative for infection only when its titres are at least two times the titres of the SAT (OIE, 2000c). This is the tests main limitation, as not all infected cattle show this ratio. The 2-mercaptoethanol and the rivanol tests detect specific IgG (MacMillan, 1990) and are usually used to differentiate between infected and vaccinated cattle.

ELISA: Several ELISA procedures and the antigens, conjugates and substrates that can be used in the assay. The ELISA has proven to be specific and as sensitive as the MRT and SAT in detecting *Brucella* antibodies in milk and serum. ELISA results are usually also in agreement with CFT results (Bercovich and Taaijke, 1990; Georgios *et al.*, 2005). The test can be used for screening and confirmation of brucellosis in both milk and serum, however, depending on the presence of traces of colostrum in the milk, or the presence of low concentrations of lacteal immunoglobulin the ELISA may test false positive or false negative (Bercovich and Taaijke, 1990; Kerkhofs *et al.*, 1990). It seems that the ELISA is less sensitive than the CFT, as some infected cattle that test positive with the CFT may test negative with the ELISA some researchers imply that the main advantage of the ELISA when compared with the CFT lies in its relative simple test procedure (Kerkhofs *et al.*, 1990). The assay is very costly when only a few samples are tested; therefore, it is unsuitable for testing individual animals but it is the ideal test for screening suspected herds.

Since the reliability of serological tests to detect brucellosis depends on antibodies that may or may not be present at the time of examination, inevitably some infected animals may elude detection. Because the skin-delayed-type-hypersensitivity (SDTH) test is independent of circulating antibodies it should be added to the serological tests to improve detection of brucellosis. The SDTH test confirms serologic test results, confirms brucellosis in cattle with ambivalent serologic test results and detects latent carriers of *Brucella*. Furthermore, the SDTH test does not sensitize cattle for several consecutive SDTH tests (Sutherland *et al.,* 1986; Bercovich, 1999). Therefore, the SDTH test should be the test of choice in developing countries, as cattle in those countries are usually not tagged so that serological test results could be related to the individual animal. Where the animals are tagged a combined use of the SAT and SDTH tests increase the reliability of brucellosis diagnosis (Bercovich, 1999; OIE, 2000c).

2.8. Prevention and control of brucellosis

2.8.1. In animals

Prevention and control of brucellosis can be adopted realistically through understanding of local and regional variations in animal husbandry practices, social customs, infrastructures and epidemiological patterns of the disease (Coetzer and Tustin, 2004). The common approaches used to control brucellosis includes, quarantine of imported stock, handling hygienic disposal of aborted fetuses, fetal membrane and discharges with subsequent disinfections of contaminated area. Animals, which are in advanced pregnancy, should be kept in isolation until parturition (Bishop *et al.*, 1994; Radostits *et al.,* 2000). Moreover replacement stock should be purchased from herd free of brucellosis, and decide for or against immunization of negative animals (Hirsh and Zee, 1999).

Immunization: Vaccines like *B. abortus* Strain 19 (S 19), which is a live vaccine and is normally given to female calves aged between three and six months as a single subcutaneous dose of 5-$8*10^{10}$ viable organisms. A reduced dose from $3*10^{8}$ to $3*10^{9}$ organisms can be administered subcutaneously to adult cattle, but some animals will develop persistent antibody titers and may abort and excrete the vaccine strain in the milk. Alternatively, it can be administered to cattle of any age as two doses of 5-$10*10^{9}$ viable organisms, given by the conjunctival route; this produces protection with out a persistent antibody response and reduces the risks of abortion and excretion in milk (OIE, 2004). The often quoted protection rate of S-19 is about 65-70% is largely based upon individual cattle challenge with standardized strains and doses. The protection on a herd basis is much greater due to reduction of clinical symptoms and increased herd resistance (Seifert, 1996).

Brucella strain 45/20 (Dyphavac) vaccine is prepared from killed rough *Brucella abortus* strain 45/20 in oil ajuvant, it lacks the smooth LPS antigen that interfere with serological diagnosis. It can be used to animals of all age and also to pregnant cows. Usually two doses of strain 45/20 vaccines 6-12 weeks apart are considered to be necessary to induce good immunity, and an annual booster is generally recommended (Seifert, 1996).

Brucella strain RB51 is as effective as one vaccination with S-19 in protecting cattle against brucellosis. It is an attenuated vaccine which essentially lacks the O-side chain of the LPS and if given singly or multiple times does not induce antibodies that interfere with conventional diagnostic tests. Several studies have reported that RB51 vaccine has proven effective for the vaccination of cattle and compatible with a control strategy based on sanitary measures alone. The official recommendation in calf hood vaccination with 10^{10} to $3\text{-}4{*}10^{10}$ colony forming units of RB51 and the suggested dose for adult animals is $1\text{-}10^{9}$ organisms. *Brucella abortus* strain RB51 has shown no tendency to revert to virulent smooth organisms after many passages in vitro or in vivo. This is probably due to the nature and place of the mutations found in this strain (Coetzer and Tustin, 2004; OIE, 2004).

Test and slaughter of positive reactors: Eradication by test and slaughter principle based on the magnitude of disease prevalence and economic status of the countries. When the seroprevalence of brucellosis is reduced to less than 2% animals which are positive to both RBPT and CFT, slaughter of positive reactors are possible (Walker, 1999; Coetzer and Tustin, 2004).

Chemotherapy: Control by treatment is mostly not successful because of the interacellular sequestration of the organisms in the lymph nodes, the mammary glands and reproductive organs. If deemed necessary the treatments often given are sulphadiazine, streptomycin, chlortetracycline and chloramphenicol (Hirsh and Zee, 1999; Radostits *et al.*, 2000).

2.8.2. In humans

The most rational approach for preventing human brucellosis is the control and elimination of the infection in animal reservoirs (Acha and Szyfers, 2001). In addition to this, there is a need to educate the:

- Public not to drink raw milk and milk products made from unpasteurized or otherwise untreated milk
- Farmers to take care in handling and disposing of aborted fetus, fetal membrane and discharges

- Abattoir workers in transmission of infection especially via conjunctiva and skin abrasions
- Laboratory workers in relation to transmission especially through contact, inhalation and accidental inoculation (Coulibaly and Yameogo, 2000).

In humans the drug recommended is rifampicin at a dosage of 600-900mg daily combined with doxicycline at 200mg daily, both drugs are given in the morning as a single dose and relapse is unusual after a course of treatment continued for at least 6 weeks (WHO, 1997).

3. MATERIALS AND METHODS

3.1. Description of the study area

The research area is located in the Western Zone of Tigray region, North West Ethiopia (Figure 1). The Western Zone is one of the 5 zones in the Tigray regional state. It has three districts, namely, Welkait, Tsegede, and Humera. The districts are located around 580-750 kms far away from Mekelle, the capital city of Tigray. Throughout the zone, livestok agriculture is the predominant economic activity with about 95% of the total population engaged directly or indirectly on it.

The geographical location of the research site is 13^0 42' to 14^0 28' North latitude and 36^0 23' to 37 ^{0}31' East longitude. Its elevation is 560 to 2800 m.a.s.l. (BOANRDT, 2003). The study area is bordered North by Eritrea, South by Gonder, East by Tahtai Adiabo and West by Sudan (Figure 1). It is a transit point for cross-border trade and traffic as well as live-animals export to Sudan and Egypt. The Western Zone is characterized by different agro-ecological zones. These include: hot to warm semi-arid low lands (Humera, and partly Welkait and Tsegede) and the tepid to cool moist mid-highlands of Welkait and Tsegede districts. Also the study area has agro-ecological zones that are hot to warm semi-arid low lands.

The annual rainfall in the area increases from North to South, which ranges 500 mm to 1500 mm per annum. Near Humera town a mean annual rainfall of 572.2mm is received while at the Southern parts of the area, the annual precipitation is estimated at about 800mm.The rainfall in the area is monomodial falling between the months May and September. Over 65% of rainfall is received in the months of July and August, while the months of November to April have virtually no rainfall (BOANRDT, 2003).

The mean monthly maximum temperature remains high throughout the year ranging from 30 ^{0}C in August to 40 ^{0}C in March and May, in the semi-arid low lands. Similarly the mean monthly temperature fluctuates very little throughout the year with the lowest 13 ^{0}C being in the month of January and highest of 35.5 ^{0}C in the month of May. The relative humidity is highest during the rainy months and lowest at the tail end of the dry season in April. The total cattle population in the three districts is estimated at 702,119. Main cattle breeds raised in the Western Zone are the local *Arado* and *Barka/ Begait* cattle. The later is the dominant breed. The *Barka* cattle breed is found in Southwestern Eritrea, Northwestern Ethiopia and South Sudan.

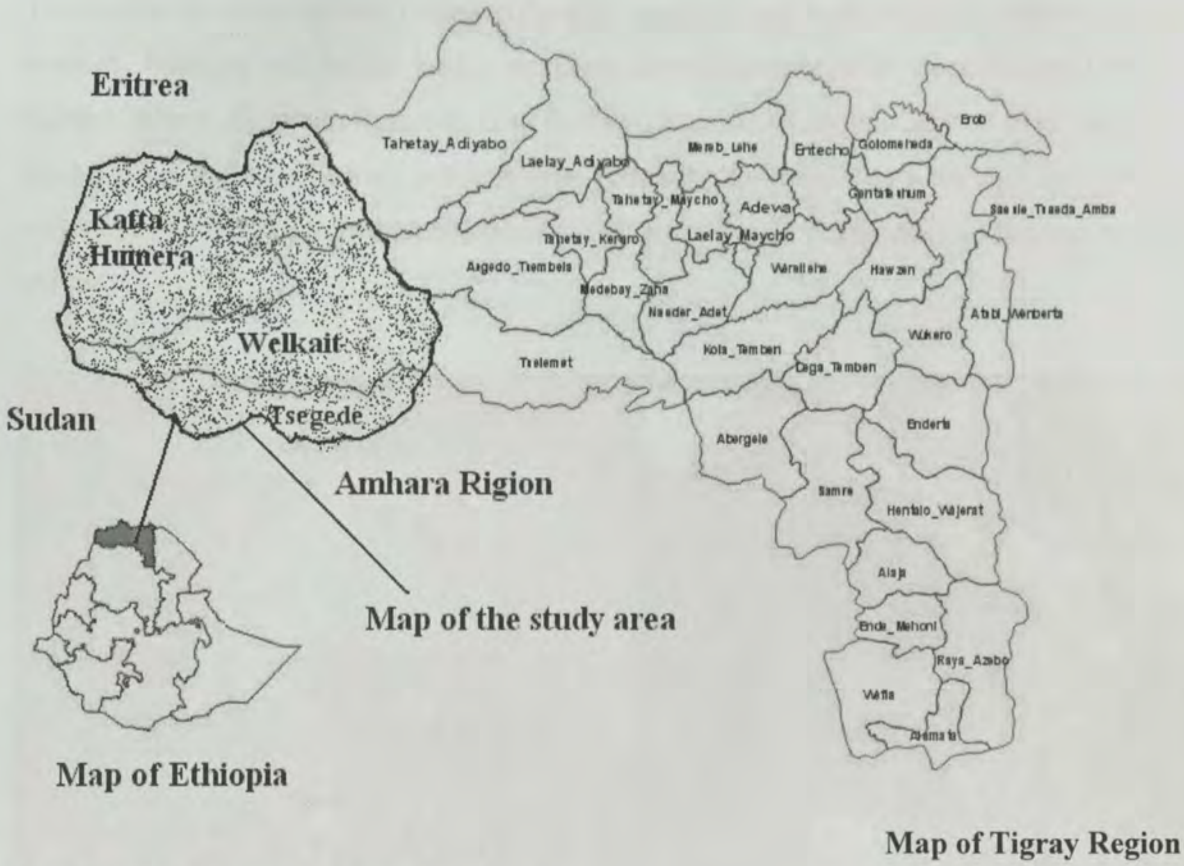


Figure 1: Map showing the study area
Source: (BOANRDT, 2003)

3.2. Study animals

The study was carried on cattle owned by smallholder farmers, were included in the study animals. The study animals comprised of local (*Barka* and non-descript zebu). *Barka*, which is the dominant cattle breed in the study area is one of the five distinguishable main cattle breed types identified in Ethiopia, used primarily for milk, meat and traction power. The breed is usually white with black spots or splashes. Occasionally red or colorsided individuals will be seen. The breed is usually horned although polled individuals are occasionally seen (Rege, 1999) (Figure 2). The true representatives of the study population for cattle were selected by combination of simple random and cluster sampling methods. Based on this a total of 1968 cattle, blood samples were collected.

The human blood sample was collected from the occupational workers (cattle attendants, abattoir workers, butchers and animal health workers) in collaboration with Zone Health Offices and Kahsay Abera Hospital. Relevant risk factors (like, removal of retained fetal membranes, drinking raw milk and contact with animals) pertaining to brucellosis were gathered during sera collection. A total of 246 human blood samples were collected purposively from those vulnerable groups.

Figure 2: *Barka* cattle breeds

3.3. Study design

A cross sectional study was conducted using questionnaire survey and serological procedures from October 2007 to February 2008 in the Western parts of Tigray region. The serological survey was intended to determine the individual animal and herd prevalence. Densities of animal populations, herd size and management, as well as environmental factors are thought to be important determinants of the infection dynamics within and between herds (Omer *et al.*, 2002). A hypothesis of this study was that the prevalence of brucellosis would be different between cattle production systems and between breeds. Pre-tested questionnaire was administered together potential risk factors that were biologically hypothesized to influence brucellosis seroprevalece. Likewise, a seroprevalence survey was conducted on potentially human risk groups for brucellosis.

3.4. Sample size determination

3.4.1. Cattle

Two types of cattle production systems were classified as extensive and semi-intensive. Sample size was determined using a method in Thrusfield (2005). Two-stage cluster sampling method was used. The extensive production system consists of mostly local *Arado* cattle and in some *Barka* breeds. The semi-intensive production system contains mainly *Barka* cattle breeds and depends on feed supplementation at home. Occasionally they are allowed to graze around the homestead and at field level. Sample size calculation was done based on Thrusfield (2005) using the following formula:

$$Ts = \frac{(1.96)^{2} * g * Pexp\,(1 - Pexp)}{g * d^{2} - (1.96)^{2} * vc}$$

Where:

g = number of clusters

d = desired absolute precision

Vc = between cluster variance

Pexp = expected prevalence

Ts = total number of animals

The study animals were selected from a total of approximately 702,119 in the Western Zone. The Zone is comprised of three districts namely Welkait, Tsegede and Humera. After having a complete list (sampling frame) of the peasant associations (PAs) in each district, 42 PAs were selected randomly from each district and included in the study. Two-stage cluster sampling technique was used where PAs and herds were the established clusters. A total of 210 clusters from the 42 PAs were selected. An average number of cattle keep in the extensive production system was 4. Thus, a total 840 cattle were designed for the sampling. An additional 8 cattle from small village (new resettlement area) were included. The number of PAs was determined using 95% confidence level, 8.2% expected prevalence (Gebreyesus, 2001) and 2% desired absolute precision (d^2). Between cluster variance was 0.0006235, which was estimated from an overall mean cluster prevalence of 8.2%. This giving a total of 848 cattle.

In the semi-intensive production system variance, between herds were not considered. All the nine towns in the district were included in the sample. After having a complete list (sampling frame) of the cattle population in the nine towns, 10% of the herds (110) and then 9 to 11 animals from each herd were randomly selected. Accordingly, in all the nine towns, 110 cattle herds and 1120 individual cattle were included in the study. This number was distributed proportionally to the nine towns. A sample size of 1120 cattle was obtained by assuming an expected prevalence of 6% reported by Mussie (2005) in Bahridar and Gonder, and a 95% confidence interval and 2% absolute precision. In the absence of between cluster variance, one can use the sample size estimated using simple random sampling method (Thrusfield, 2005). This sample size is then inflated by 2 to 4 folds to account for the potentially large variation that occur among clusters. In this study two fold was used, and a sample size of 1120 was obtained.

3.4.2. Sample size for Public health survey

Blood examination was carried out in collaboration with Kahsay Abera Hospital. The sample size for estimating the seroprevalence of brucellosis in risk groups was determined by standard procedure as described in Thrusfield (2005). The following formula was used.

$$n = \frac{1.96^2 * Pexp\ (1-Pexp)}{d^2}$$

Where

n = required sample size

Pexp = expected prevalence

d = desired absolute precision

An expected prevalence of 20% and 5% desired absolute precision were used and a sample size of 246 persons was obtained.

3.5. Data collection

Between August and September 2007 were animal inventory, site assessment and an extension system for the farmers were made. Blood sampling and administration of the questionnaire survey were performed during the regular visits to each peasant associations and towns between October, November, December, January and February, 2007/2008. The randomly selected livestock owners from the semi-intensive and extensive production system were interviewed using a pre-tested structured questionnaire to determine management and husbandry risk factors, which were known or thought to influence the spread and maintenance of brucellosis. Reproductive parameters are presumed to be affected by brucellosis were also incorporated in the questionnaire (Annex 1). Generally, the blood samples and questionnaire data were labeled by each town and peasant associations in each district.

In case of humans all the vulnerable groups assumed to be testing for brucellosis were interviewed related to risk factors (animal health workers, butchers, abattoir workers and herdsmen) (Annex 2).

After collecting blood sample, laboratory analyses were done at Mekelle Regional Laboratory in Tigray Regional State and National Veterinary Institute, NVI, Debre Zeit. The respondent recall memory was used as a data source. This result was then compared with serological results.

3.5.1. Blood collection

About 10 ml of whole blood sample was collected from the jugular vein using plain vaccutainer tubes and needles from each animal aged above six months and with no history of vaccination for brucellosis. Each sample was labeled using codes specific to the individual animal and herd information. The tubes were tilted on a table overnight at a room temperature to allow clotting. Serum was collected either passively by decanting or using centrifuges at 2500 rpm for 5 minutes. The serum was stored at -20 ^{0}C until it was tested by Rose Bengal Plate Test and Complement Fixation Test.

Blood sampling in humans were carried out by professional medical team from Humera Kahsay Abera Hospital. A total of 246 human blood samples were obtained from people in high-risk groups (animal health workers, butchers, abattoir workers and herdsmen) who had contacts with and consumed animals and animal products, respectively. Approximately 5 ml of blood was collected from each member in the risk groups. The processing, preservation and testing followed same procedures as described for animal blood samples in this paragraph.

3.7. Serological detection of *Brucella* antibody

3.7.1. Rose Bengal Plate test (RBPT)

The RBPT is a rapid agglutination test that uses stained antigen at a pH of 3.65. It helps to visualize the results and eliminates some non-specific reactivity. Generally, equal amounts of antigen and serum was mixed and agglutination was graded as N, 1, 2, 3+ depending on the number of agglutinated cells. The low pH of the assay prevents agglutination with IgM and agglutinates only IgG1. This reduces non-specific interactions. Serum samples from both cattle and humans were screened using the standard Rose Bengal plate test (RBPT), employing stained *B. abortus* antigen (INSTITUTE POURQUIR, 326, rue de la galera 34097 MONTPELLIER CEDEX 5, France) and known positive and negative reference sera.

B. abortus antigen was heat inactivated and 0.5% phenol adjusted to pH 3.65 and colored with Rose Bengal. The test was undertaken at Mekelle Regional Laboratory; Tigray Regional State.

The RBPT method prescribed by BgVV Service Laboratory (2000) Berlin, Germany was followed briefly as follows:

The reference and test sera, and antigen were left at room temperature for half an hour before testing then,

- 30 μl of serum on each square of the plate /microscopic slide was added;
- 30 μl of antigen was placed along side the serum;
- The antigen and serum was mixed thoroughly with applicator stick;
- The plate was rocked by hand for 4 minutes;
- The test was read by comparing with the positive and negative control sera by examining for agglutination in natural light;
- Magnifying glass was used to detect micro-agglutination.

The interpretation was performed as described by Staak *et al*. (2000) was followed:

(N) 0: No agglutination

+: Barely precipitable

++: Fine agglutination, some clearing

+++: Course clamping, definite clearing

Those samples identified with no agglutination were recorded as negative, those with +, ++, and +++ were recorded as positive.

3.7.2. Complement Fixation Test (CFT)

Although it is complex to perform, requiring good laboratory facilities and adequately trained staff to accurately titrate and maintain the reagents, it is widely used and accepted confirmatory test. The CFT procedure was undertaken at the National Veterinary Institute Department of Immunology, Debre-Zeit. Preparations of the reagents were performed according to OIE protocols (OIE, 2000c; Annex 4). Briefly the procedure of the test was carried stepwise as follows:

- All the wells of Micro titer plates were filled with test sera and incubated at 58 ^{0}C in water bath for 30 minutes in order to inactivate the naive complement;
- A dilution of each serum was made at 1: 5 with veronal calcium and magnesium;

- 25 µl of each diluted test serum was placed in wells of first and second rows of U-bottom plate, and 25 µl of veronal buffer was added to all wells except those of the first row;
- Serial doubling dilutions were then made by transferring 25 µl volume of sera from 2nd row on wards for at least 4 dilutions;
- 25 µl of antigen diluted at a working dilution of 1:20 excluding those of anti-complementary controls, received 25 µl of VCM;
- 25 µl of complement at working dilution of 1:10 was added to all wells except control wells;
- Control wells containing serum control had serum + complement + diluents and antigen control had antigen + complement + diluents. Complement control well had complement + diluents and, hemolytic system had diluents set up to contain 75 µl total volume in each case before hemolytic system was added;
- The plates were incubated for 30 minutes at 37 ^{0}C (warm fixation);
- 25 µl of 2% SRBC and amboceptor (hemolytic system) mixture was added into all the wells;
- Plates were sealed with tapes and placed on a shaker and incubated at 37 ^{0}C for 30 minutes;
- Before reading the result the plates were left in a refrigerator at + 4 ^{0}C for an hour in order to allow non-lysed cells to settle;
- Plates were taken out from refrigerator and results were read after being left on the table for 10 minutes at room temperature;

As for the interpretation of test results, Positive reactions were indicated by sedimentation of SRBC and absence of hemolysis. Negative reactions revealed by hemolysis of SRBC. According to OIE (2004) sera with strong reaction, more than 75% fixation of complement at a dilution of 1:10 and at least with 50% fixation of complement at a working dilution (1:5) was classified as positive.

3.8. Data management and analyses

Data obtained from both serological tests and questionnaire survey were stored in Microsoft excel spreadsheet (Microsoft Corp.). These data were analyzed by descriptive statistics, univariate and multivariate regression using the SPSS 11.5 statistical package (SPSS, 2002). Animals and humans teste positives to both RBPT and CFT were defined as seropositive. Clusters (herds) having at least one seropositive cattle were considered positive for brucellosis. The individual animal and human level seroprevalence was calculated on the basis of RBPT and CFT positive results divided by total number of animals and humans tested. Similarly, herd (clusters) level seroprevalence was computed as the number of clusters (herds) with at least one positive animals divided by the total number of clusters (herds) tested.

Questionnaire data that included risk factors associated with husbandry management systems like, females having infertility problems, whether sold or kept within herds (reasons for sell of breeding cows), method of disposal of fetal membranes, dry season watering point and those reproductive parameters thought to influence the disease like, calving intervals were administered and compared with that of serological results.

The Chi-square test was applied to determine existence of any association between seropositivity and some risk factors in cattle and associations between human positivity and contacts with animals. To measure the strengths of the associations univariable logistic regression was applied to calculate Odds ratio. Moreover to see the effect of some factors on a dependent or response variable that may be influenced by the presence of other factors through effect modifications (i.e. interactions), all the significant variables from univariable logistic regression was further analyzed by multivariable logistic regression. $P<0.05$ was taken as significant.

4. RESULTS

4.1. Seroprevalence of brucellois in cattle

A total of 1968 bovine sera were examined from Western Zone of Tigray Region. The overall seroprevalence of brucellosis in the study population was 114 (5.8%) and 96 (4.9%), under RBPT and CFT test respectively. Seroprevalence of the disease was almost comparable in the nine towns of the semi-intensive production system (Table 6). A total of 86 (7.7%) *Brucella* seropositive was found from the 1120 tested cattle. Moreover district level seroprevalence, in individual and cluster, were also determined under extensive production system. A total of 10 (1.2%) seropositive to *Brucella* antibodies were found from 848 individual cattle and 7 (3.3%) from 210 animal clusters. To measure the strength of the relationships between seropositivity and each districts logistic regression analyses was performed to determine the odds ratio, there were no significant associations between seropositivity to brucellosis and districts in both individual and cluster of animals (Table 7).

Table 6: Distribution of the overall individual cattle *Brucella* seroprevalence in nine towns under semi-intensive production system in Western Zone, Tigray Region (October, December, 2007 and January to February, 2008)

					95%CI of OR	
Town	n*	Prevalence (%)	P- value	OR	Lower	Upper
Tirkan-Aidola	89	4.5	0.850			
Adigeshu	63	4.8	0.938	1.062	0.229	4.922
Humera	17	5.9	0.805	1.328	0.139	12.670
Hagereselam	33	6.1	0.723	1.371	0.239	7.862
Baeker	264	6.8	0.436	1.555	0.512	4.723
Adebay	129	7.8	0.341	1.786	0.542	5.884
Rawian	25	8	0.494	1.848	0.318	10,727
Bereket	144	8.3	0.405	1.932	0.410	9.102
Mykadra	356	9.6	0.148	2.170	0.759	6.207
Total	1120	7.7				

n* =Total number of animals tested

Table 7: Distribution of individual and cluster *Brucella* seroprevalence in cattle under extensive production system of Western Zone Tigray Regional state (October, December, 2007 and January to February, 2008)

Districts		Individual level			Cluster level			
	n*	Prevalence (%)	P- value	95% CI for OR	n**	Prevalence (%)	P- value	95 %CI for OR
Welkait	220	0.91	0.236		55	3.6	0.326	
Tsegede	528	0.95	0.961	1.042 (0.201, 5.412)	132	2.3	0.602	0.616 (0.100, 3.794)
Humera	100	3.00	0.187	3.371 (0.554, 10.499)	23	8.7	0.370	2.524 (0.333, 19.103)

n* =Total number of individual animals tested

n**= Total animal clusters tested

Differences and associations of factors affecting seroprevalence of bovine brucellosis

The differences between cattle seroprevalence of brucellosis per each risk factor categories as well as associations are summarized in table 8. Animal production system was classified into extensive and semi-intensive ones to measure the association with seropositivity of the infection. The result indicated that seropositivity to *Brucella* antibodies in semi-intensive production system was significantly (P=0.000) higher than in the extensive one.

Herd size was also categorized into three groups, 1-5, 5-10 and >10 cattle, to assess the association with seropositivity of the infection. There were significant (P=0.000) differences among the herd sizes with higher seroprevalence of brucellosis in large herd sizes.

Comparison was undertaken in both *Arado* and *Barka* breeds and there was statistically significant different (P=0.000) between the two breeds. *Barka* breeds were demonstrated higher seroprevalence of 7.2% compared to *Arado* of 1.3%.

The ages of animals were also classified into three categories to assess an association of brucellosis in different age groups. These age groups were of 0.5-3 years, 3-6 years and >6 years.

Thus the univariable logistic regression model showed that ages above 6 years had significant impact on animal seropositivity to brucellosis (P=0.005).

Sex has supposed some association with the epidemiology of brucellosis. Comparison was made on the seroprevalence both male and female breeds there was statistically significant different between the two sexes were observed (P=0.017). Female cattle demonstrated higher seroprevalence (Table 8).

During the statistical analyses in all risk factors the first level of each independent variable was used as a reference category.

Table 8: Summary results of the univariate logistic-regression analyses of risk factors with dependent CFT *Brucella* seropositivity in cattle in Western Zone of Tigray Regional State

Risk factors	Category Levels	n*	Prevalence (%)	P- value	OR	95% CI of OR	
						Lower	Upper
Production system	Extensive	848	1.2				
	Semi-intensive	1120	7.7	0.000	6.970	3.598	13.501
Breed	*Arado*	768	1.3				
	Barka	1200	7.2	0.000	5.852	3.021	11.337
Sex	Male	446	2.7				
	Female	1522	5.5	0.017	2.113	1.143	3.905
Age	<3years	702	3.4	0.017			
	3-6years	822	4.9	0.162**	1.445	0.862	2.422
	>6years	444	7.2	0.005	2.194	1.275	3.777
Herd size	<5cattle	351	1.1	0.000			
	5-10cattle	721	3.1	0.067**	2.730	0.934	7.985
	>10cattle	896	7.8	0.000	7.352	2.663	20.293

n* = Total number of animals tested

** = No significance differences at $P<0.05$

The multivariable logistic regression model (Table 9) showed that production system, sex, age and herd size were significantly associated cattle seropositivity to *Brucella* antibody. However no statistically significant association (P=0.070) was observed between breeds and seropositivity. Nevertheless, OR=2.130 was greater than one.

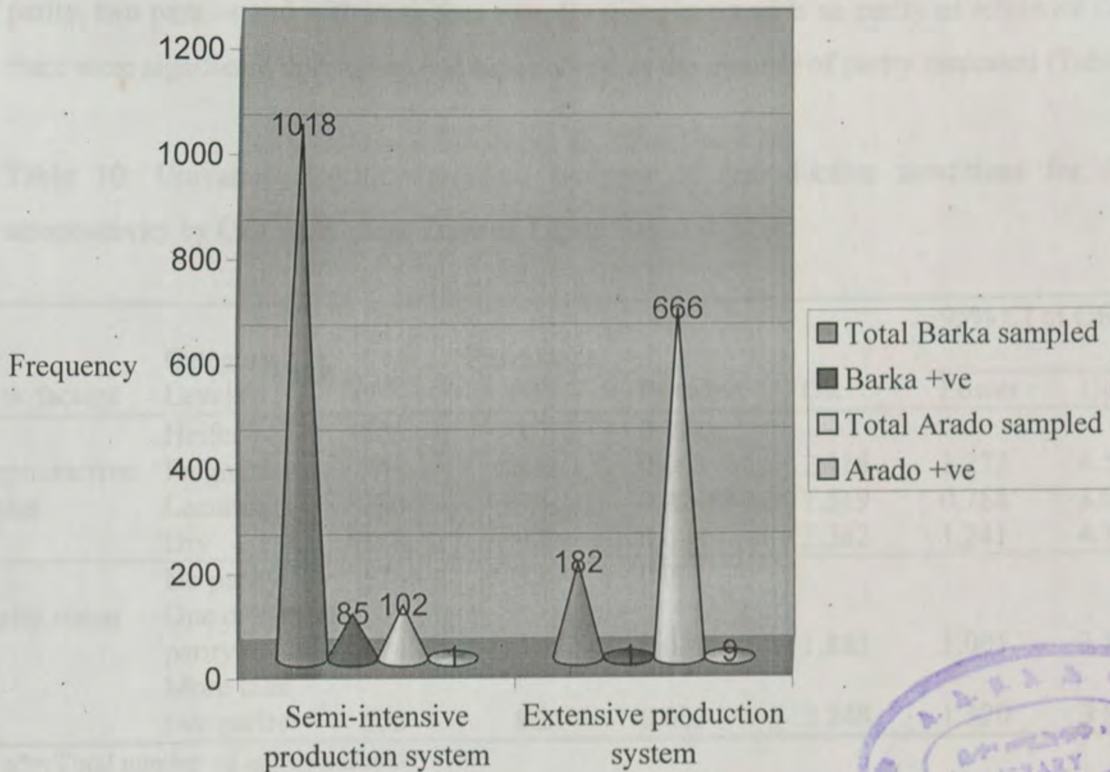
Table 9: Multivariable logistic regressions of risk factors for *Brucella* seropositivity by CFT in Western Zone of Tigray Regional State

Risk factors	Estimated Coefficient	S.E.	P- value	OR	95% CI of OR Lower	95% CI of OR Upper
Production system	1.218	0.418	0.004	3.382	1.492	7.666
Breed	0.756	0.418	0.070	2.130	0.939	4.832
Sex	0.707	0.318	0.026	2.029	1.088	3.782
Age	0.355	0.141	0.012	1.427	1.082	1.881
Herd size	0.700	0.194	0.000	2.014	1.377	2.946
Constant	-6.452	0.542	0.000	0.002		

S.E = Standard Error of the coefficient

Breed susceptibility to brucellosis under the same production system

To see breed susceptibility differences to brucellosis, both *Arado* and *Barka* breeds kept under the same production system (semi-intensive type) were examined. The result indicated 1% (n=102 *Arado* cattle) and 8.3% (n=1018 *Barka* cattle) were seropositive. This wide variation was statistically significant (P=0.003). Furthermore, in the extensive production system 1.4% (n= 666 of *Arado* cattle) and 0.5% (n=182 *Barka* cattle) were obtained. This result was not statistically significant (P 0.698; Figure 3).



+ve=positive to *Brucella* antibody

Figure 3 : Comparison of CFT seropositive cattle breeds to brucellosis under extensive and semi-intensive production system

Reproduction conditions and seropositivity to *Brucella* antibody

An attempt was made to find out the association of the seropositivity with different reproductive status. Females were categorized into four groups: heifer, pregnant, lactating and dry (Table 10). By Using heifer as reference category, univariable logistic regression analyzes of the seropositivity to *Brucella* antibodies in pregnant and dry cows significant differences and association were observed (P<0.05) except for lactating of cows.

Cattle at different parity numbers were also grouped into three categorizes as cows with no parity, two parities and with more than two. By using cows with no parity as reference category, there were significant differences and associations as the number of parity increased (Table 10).

Table 10: Univariate logistic-regression analyses of reproductive conditions for *Brucella* seropositivity by CFT in Western Zone of Tigray Regional State

Risk factors	Category Levels	n*	Prevalence (%)	P- value	OR	95% CI of OR	
						Lower	Upper
Reproductive status	Heifer	506	3.7	0.021			
	Pregnant	244	8.6	0.007	2.414	1.272	4.580
	Lactating	286	5.6	0.229**	1.519	0.768	3.003
	Dry	318	8.2	0.008	2.282	1.241	4.196
Parity status	No parity	578	3.8	0.011			
	One or two parity	274	6.9	0.049	1.883	1.001	3.541
	More than two parity	502	8.2	0.003	2.248	1.320	3.828

n*= Total number of animals tested

**= No significance differences and associations at P<0.05

The multivariable logistic-regression model (Table 11) showed that parity status had significant (P=0.042) association cattle seropositivity to *Brucella* antibody. However, there was no significant (P=0.991) association with reproductive status.

Table 11: Multivariate logistic regression of reproductive conditions for *Brucella* seropositivity in Western Zone of Tigray Regional State

Risk factors	Estimated Coefficient	S.E.	P- value	OR	95% CI of OR	
					Lower	Upper
Reproductive status	-0.009	0.148	0.953	0.991	0.742	1.324
Parity status	0.399	0.197	0.042	1.491	1.014	2.192
Constant	-3.160	0.204	0.000	0.042		

S.E = Standard Error of the Coefficient

Seroprevalence of Brucellosis and stages of Pregnancy

Pregnant cattle (n=244) were categorized into two groups on the basis of their stage of gestation. The first group comprised of cattle below five months of pregnancy (n=146) and the other group having cattle with equal to and above five months of pregnancy (n=98). Higher proportion of 12.2%, (n=12) *Brucella* seropositivity was found in pregnant cows of above or equal to five months than cattle below five months of pregnancy of 6.2% (n=9) (Figure 6).

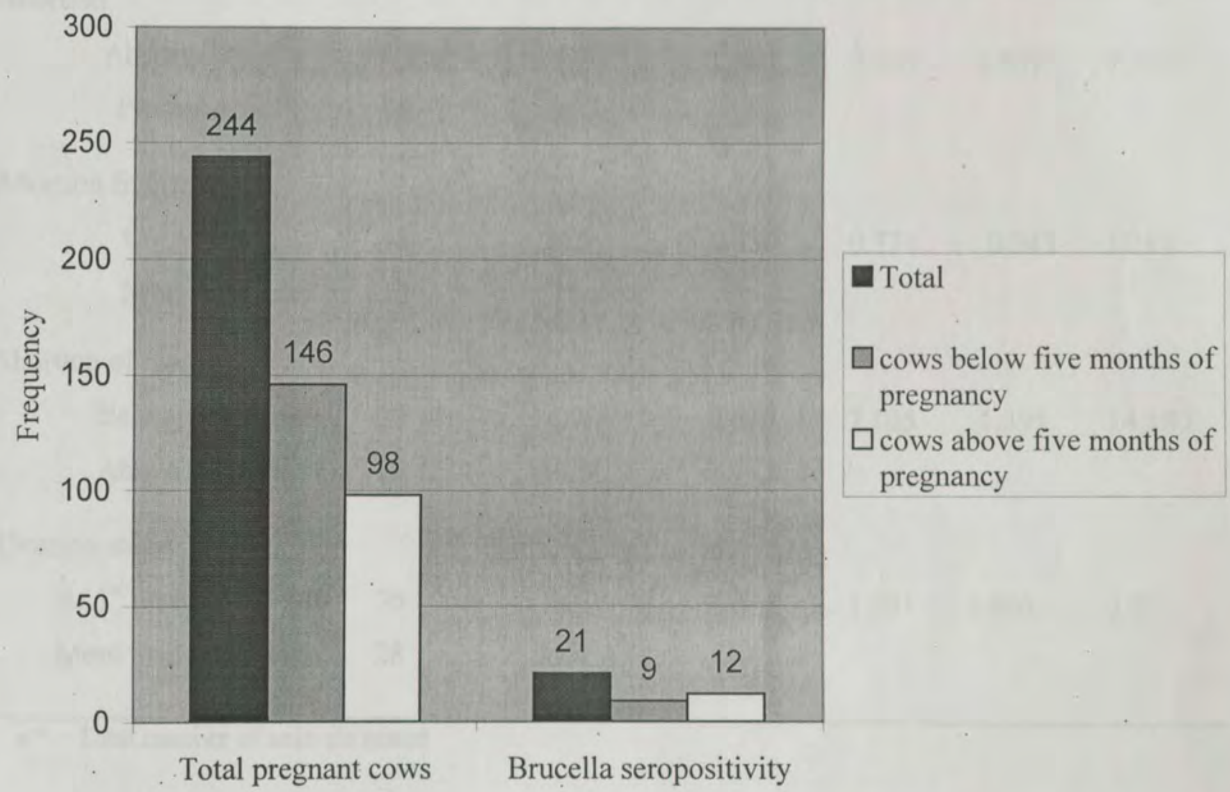


Figure 4: Comparison of CFT *Brucella* seropositivity of cows above and below five months of pregnancy

Association between presence of *Brucella* antibodies and history of abortion

Since brucellosis is an important cause of abortion in cattle (Chukwu, 1987), an attempt was made to correlate a previous history of abortion, abortion frequency (only once and more than months and below five months) with seropositivity due to brucellosis (Table 12). Statistically significant differences and associations were observed between seropositivities by absence and presence of abortions (P=0.001; OR=3.641).

Table 12: Associations between CFT *Brucella* seropositivity and history of abortion in cows in Western Zone of Tigray Regional State

Status of abortion	CFT test				95%CI of OR	
	n*	Prevalence (%)	P-value	OR	Lower	Upper
Abortion						
Absent	718	6.7	0.001	3.641	1.809	7.330
Presence	58	20.7				
Abortion frequency						
Only once	48	22.9	0.359	0.374	0.043	3.283
More than one	10	10				
Abortion of month						
Below five month	29	6.9	0.010	7.105	1.395	14.180
Above five month	29	34.5				
Abortion stage						
At 1st parity	30	20	0.893	1.091	0.306	3.888
More than one parity	28	21.4				

n* = Total number of animals tested

Questionnaire data on management risk factors and some reproductive parameters and their associations with CFT *Brucella* seropositivity

One hundred ten and sixty-two randomly selected livestock owners were interviewed in the semi-intensive and extensive production systems respectively to correlate the associations of the management and husbandry risk factors with serological results. From the questionnaire and test result in the extensive production system no positive results were recorded. In contrast, in the semi-intensive production system, it was observed that 24.5% (n=27) of the respondants had seropositive cattle. Among the management and husbandry risk factors less *Burcella* antibody seroreactors were revealed (3.1%) from those who sell and slaughter cattle with infertility problems than those who kept with the herd (32.9%; Table 12). Similarly, *Brucella* antibody seroprevalence on herd reproductive performance was conducted by comparing serological results with questionnaire data (Table 13) and significant difference and an association between CFT seroprevalence with calving interval above fifteen months and at least fifteen months were observed (P= 0.002; OR=4.964).

Table 13: Ccomparisons of management and husbandry risk factors and the questionnaire data with serological result in the semi-intensive production system

Management husbandry risk factors	CFT test				95% CI of OR	
	n*	Prevalence (%)	P- value	OR	Lower	Upper
Grazing system						
Individual	67	22.4	0.512	1.342	0.557	3.235
Communal	43	27.9				
Culling method (Cattle having infertility problem)						
Sell and slaughter	31	3.1	0.001	14.717	1.901	28.964
Keep with their herd	79	32.9				
Watering point						
Wells	32	6.3	0.003	7.075	1.566	31.974
River	78	32.1				
Disposal of fetal membrane						
Burring	21	14.3	0.272	2.215	0.598	8.201
Throwing to the field	89	27.0				

n* = Total number of animals tested

Table 14: Impact of *Brucella* antibody seroprevalence on cattle reproductive performance comparing serological results with questionnaire data in the semi-intensive production system

Reproductive parameters	CFT test				95% CI of OR	
	Total cattle tested	Prevalence (%)	P-value	OR	Lower	Upper
Calving interval						
<15 months	49	10.2	0.002	4.964	1.716	14.363
>/=15 months	61	36.1				

4.2. The seroprevalence of brucellosis in humans

Of the 246 human serum samples collected, 21 from females and the rest were from males. No *Brucella* seroreactive case was identified from females. Four of the positive sera to RBPT were from males. The RBPT positive sera were further examined by CFT. Three were found seropositve for *Brucella* antibody. All the three were from herdsmen (Table14). Analysis of questionnaire results indicated that, there was significant difference between removed retained fetal membrane and prevalence of brucellosis (P=0.046). Moreover herdsmen who drank raw milk of 2.5% and animal contacts of 1.3% seroprevalence were found (Table 15).

Table 15: Seroprevalence of CFT *Brucella* seropositivity in different risk groups of people in Western Tigray (October, December, 2007 and January to February, 2008)

Vulnerable groups for brucellosis	Number of sample		Prevalence (%)
	Males	Females	
Animal heath workers	7	-	0
Butchers	21	8	0
Abattoir workers	12	-	0
Herdsmen	185	13	1.5
Total	225	21	1.2

Table 16: Association of human CFT *Brucella* seropositive with some risk factors in Western Tigray

Risk factors	Categories	CFT test result		P- value
		Total human sampled	Prevalence (%)	
Removed of retained fetal membrane	No	157	0	
	Yes	89	3.4	0.046
Drinking raw milk	No	124	0	
	Yes	122	2.5	0.120
Animal contacts	No	11	0	
	Yes	235	1.3	0.871

No 95% CI, because some contingency table cell had zero observations.

5. DISCUSSIONS

Overall prevalences of bovine brucellosis

Seroprevalence of bovine brucellosis at different times in different corners of the country have been established (Table 4). In the current study by CFT test 4.9% seroprevalence of bovine brucellosis were found in the western zone of Tigray. This proportion considerably higher than most of those reported in previously in the region. Aberha (2003) and Gebretsadik (2005) reported 0.69% and 1.49% of bovine brucellosis respectively in the region. In contrary to the findings of the present study seroprevalences as large as 22% in Chafa State Farm (Sintaro, 1994), 19.5% in Abernosa Cattle Breeding Ranch (Taye, 1991), and 15.8% in Sidamo (Endrias, 1989) were reported outside this region. On the other hand, 4.63% (Mussie, 2005), 4.9% (Abay *et al.,* 2000), 4.2% (Tekleye *et al*., 1989) were reported. Comparatively these agree with the present findings. Comparable seroprevalences were also reported in other African countries as follows: 4% in Djibouti (Chantal *et al.,* 1996), 3.3% in CAR (Nakoune *et al*., 2004), 5% in Sudan (El-Ansary *et al.,* 2001) and 4.2% in nomadic pastoral cattle of Eritrea (Omer *et al.,* 2000).

According to Acha and Szyfers (2001) the rates of infection vary greatly from one country to another and also within a country. The present findings showed variations among the three study districts: Tsegede (0.95%), Humera (3%) and Welkait (0.91%) were observed under the extensive production system. But statistically significant differences were not observed among the nine towns located in the semi-intensive production system area; nevertheless, Proportionally higher seropositive to brucellosis was found in Mykadra town compared to other towns (Table 6). The reason for high prevalence was probably due to the fact that Mykadra town is located to Sudan and Eritrea border at which freely animal movement is common from country to country. This is also true that mixing of animals from different areas can facilitate spread of the disease between an infected herd and *Brucella* susceptible free herds (Kubuafor *et al.,* 2000; Menachem, 2002).

Radostits *et al.* (2000) reported unexpectedly significant higher seropositivity to brucellosis in animals in hotter lowlands of agro-climate, which they described as unsuitable for survival of *Brucella* organisms. However, this could be probably show that agro-climate is not a crucial risk factor for occurrences of brucellosis but may have a confounding effect with other management factors. In the current study, 8.3% of the seroprevalence was recorded in *Barka* breeds in the semi-intensive production system in the hot lowlands of Humera. Contrary Tekleye *et al.* (1989) reported 4.3% of seroprevalence in lowlands of Gibe.

Host risk factors

According to Walker (1999) susceptibility to infection depends on production system, herd size, breed, age and sex of the animal. The obtained production system specific seroprevalences were 1.2% and 7.7% for extensive and semi-intensive systems respectively. They were significantly different (P=0.000). The low seroprevalence in the extensive production system might be due to the fact that most cattle of the new settlers were under the extensive production system whose animals were from other districts in which low seroprevalence of brucellosis of 1.49% (Gebretsadik, 2005) and 0.69% (Aberha, 2003) had been reported. Herd sizes in this production system were small and livestock owners used individual watering points, which greatly might have contributed the spread of the disease. This hypothesis is supported by Crawford *et al.* (1990) who described that management practices of herds have great contributions to the occurrence of infection within a herd. Furthermore, Abay *et al.* (2000) reported a seroprevalence of 2% and Kassahun (2004) a seroprevalence of 1.66% in the extensive production systems that are near to the findings in this study. On the other hand higher seroprevalence in the semi-intensive production system have been revealed. Mussie (2005) got a high seroprevalence in semi-intensive of 5.97% compared with that in the extensive production of 3.82%. Weidman (1991) reported that the rise of infection with change from extensive to intensive management might be due to the close correlation between the intensities of husbandry practices and rates of infection.

Crawford *et al.* (1990) and Omer *et al.* (2002) stated that stocking densities are important potential determinants between susceptible and infected animals. This concept coincides with the current study that the seroprevalnce of brucellosis among three categorized herd sizes, < 5 cattle, 5 -10 cattle and >10 cattle, Significant variations (P=0.00) were found with higher seroprevalence was recorded in the large herd size. This is due to presence of easily contact between infected and susceptible animals. The significantly higher seropositivity in the large herd size categories is in concordance with several authors, large herd size was reported as one of the major risk factors for occurrence high prevalence of bovine brucellosis (Hellman *et al.*, 1984; Miga *et al.*, 1996; Yilkal *et al.*, 1998; Tadele, 2004; Mussie, 2005 and Muma *et al.*, 2007).

An attempt was also made to compare the seropositivity rates between *Arado* and *Barka* cattle. Higher seroprevalence with significant difference (P=0.000) was revealed in *Barka* breeds in the univariate regression analyses but due to some confounders no difference (P=0.070) was observed in the results of adjusted multivariate regression analysis. An assessment was also conducted within breeds raised in the same and different production systems. Apparently 1% of *Arado* and 8.3% of *Barka* breeds of cattle were found seropositive to *Brucella* antibody in the semi-intensive production system, which were significantly (P=0.003) different. However, no statistically (P=0.698) significant difference was recorded between the two breeds in the extensive production system. The difference was found only in cattle breeds that grouped together in the semi-intensive production system. This could be due to large herd sizes, effect on husbandry and management practices. In addition, introduction of infected animals from neighboring countries can affect the magnitudes of seroprevalence as reported by Crawford *et al.* (1990). In comparison to the present study, Jiwa *et al.* (1996) reported significant (P<0.001) variations among Tanganyika shorthorn zebu and Grade animal (zebu and exotic), Mpwapwa and exotic animals in Victoria province of Tanzania. Conversely, Kubuafor *et al.* (2000) found no significant differences among three types of breeds (Sanga, West Africa short horn and White Fulani) in Ghana.

Age categorization was made to assess an association of the seroprevalence with age. Despite the increment in seropositivity with age, no significant differences were observed between age <3 years and 3-6 years. However higher seropositivity of 7.2% in above 6years age group followed by 3-6 age group with a 4.9% and by <3 age group with a 3.4% was observed in the study. Overall in three-age groups significant differences (P=0.012) were observed. This finding is consistent with Sintaro (1994) found higher proportions of seroprevalence of brucellosis in old cattle. The rise of infection in advances by age agrees with report of Yilkal *et al.* (1998), Abay *et al.* (2000) and Omer *et al.* (2000). Furthermore, Kubuafor *et al.* (2000) reported higher seropositivity with respect to age in Ghana. Walker (1999) described that Younger animals tend to be more resistant to infection and frequently clear infections than sexually mature animals.

Regarding to sex, higher number of seropositive females were found in the study area. Moreover the apparently high seroprevalence figure in females (5.5%) than males (2.7%) agrees with the findings of Abay *et al*. (2000), Tadele (2004) and Gebretsadik (2005). Similarly, Nicoletti (1984) stated that males are more resistant than females.

Reproductive status

Among the four categorized reproductive status, heifers, pregnant, lactating and dry cows, only small seroreactors were recorded in heifers. This is in agreement with Radostits *et al*. (2000) who reported that sexually mature and pregnant cattle are more susceptible to infection with the organism than sexually immature cattle of either sex. Omer *et al.* (2002) and Mussie (2005) also reported comparable findings where high seroprevalence was found in adult cows irrespective of their lactating or pregnancy status. Moreover, relatively high proportions of seroreactor were found in pregnant cows (Table7). The higher seropositive to *Brucella* antibody in cows above five month of pregnancy could be due to the preferential localization of *Brucella* in the uterus in which allantoic fluid factors such as erythritol, a four-carbon alcohol could stimulate the growth of *Brucella* and elevate in the placenta and fetal fluid from about the fifth month of gestation (Walker, 1999; Bishop *et al*., 1994). However, there is a reduction in the number of organisms found in the months following calving and abortion, and cows usually eventually become non-infective until the next pregnancy when there is again a rapid increase of *Brucella* organisms in the reproductive tract (Coetzer and Tustin, 2004).

The associations and seroreactor rates among three parity stages (no parity, one and two parity and more than two parities) were performed. A significant association was found among parity numbers and seropositivity to *Brucella* antibodies (P=0.042). This could be directly related to sexual maturity of the animal. Radostits *et al*. (2000) showed that significant differences exist between animals with no birth tended to be more resistant to infection. In consistence with the present study, Mussie (2005) found significant associations between seropositivity and semi-intensive production systems and Yilkal *et al.* (1998) found a significant association between animals having at least one parturation.

History of abortion and seroprevalence of brucellosis

John *et al.* (2002) reported that for bovine brucellosis, the major direct losses on reproduction were abortion and impaired fertility. In the current study, among the abortions a significant association (P=0.001) was found with seropositivity of the infection. These are supported by report of McDermott, *et al.* (1987) in Southern Sudan who reported abortion rates of 22% in seropositives versus 11% in seronegatives. Tadele (2004) also found cattle with history of abortion were at higher odds of being seropositive compared to those with history of abortion in Jimma zone. Yilkal *et al.* (1998) also found significant association between abortion and seroreactors to *Brucella* at herd level. Cows that abort at least once as well as cows that abort at the first parity and more have no any significant difference with seropositivities to brucellosis. Contrary to these findings, cows that abort below five months and above five months of pregnancy were found significantly associated (P=0.000) with seropositivity of the disease. This agreed with Acha and Szyfers (1989) who showed that predominant symptoms in pregnant females are late abortion and premature or full term birth of dead or weak calves

Management and husbandry risk factors and impact of the disease on some reproductive parameters

Considering the contagious nature of *Brucella* species, sharing grazing land and drinking water facilitate transmission of the disease (Jiwa *et al.,* 1996). This is in agreement to the current study where one hundred and ten randomly selected cattle owners in the semi-intensive production system were interviewed and a statistically significant (P=0.001) between those who had cattle having infertility problems were observed. This result was common with the habit of farmers in the area who kept cattle for prestige without selling and slaughtering. This could have increased the risks of disease transmission. Walker (1999) indicated brucellosis transmission was high when infected animals were found in the herd. Moreover, farmers that used individual wells, as watering points had lower proportions of seroreactors than farmers that used communal ones. This is compatible with Muma *et al.* (2007) who reported animals on the plains often grazing and watering along with the waterfront standing in water up to 50 cm in depth since *Brucella* organisms can survive in water (at 20 ^{0}c for 2.5 months), sharing infection through environmental contamination was likely both within and across animal species. This is true reported in Bahrdar (Mussie, 2005) who reported an association between husbandry risk factors and seroprevalence of *Brucella* antibodies. In the study area, farmers that disposed the fetal membrane to the fields and gave to dogs as well as those who used communal grazing system were found to have high proportion of seroreactors.

Brucellosis causes heavy economic loses in livestock producers that stems from abortion, losses of calves due to abortion and increasing calving interval (Georgios *et al.*, 2005). This is consistent with the present study. Seropositive cows had relatively longer calving intervals than the seronegative ones.

In humans

Seroprevalence rates of *Brucella* antibodies can be very high at particular among populations in endemic areas. The prevalence of brucellosis in man is largely influenced by the pervalence of disease among domestic animals around him (Mohamed, 2002; Omer *et al.*, 2002). At the present study only 1.2% (n=3) of human sera were seropositive to *Brucella* antibody. Among the risk groups tested, abattoir and animal heath workers, butchers and herdsmen analyzed seroprevalence to *Brucella* antibody was found only among herdsmen. This findings of seropositivity in humans agrees with Mussie (2005) in Bahridar, Kassahun (2004) in Sidamo zone and Tadele (2004) in Jimma zone. This is due to the consumption of unpasteurized or unboiled milk a common practice among the herdsmen. This is also true in Oman (Mohamed, 2002) among the 375 cases of brucellosis ingestion of raw milk and its products were responsible for the disease in 63% of cases. Generally bucellosis seroprevalence is high in risk groups and in others very low or zero in groups that are not at risk due to profession, lifestyles or food eating habits. For instance seroprevalence of brucellosis was found 14% in abattoir workers and zero for blood donors, and 3.8% in nomadic pastoralists in Chad (Massenet *et al.,* 1993), 6.5% in slaughterhouse workers in

Djibouti (Chantal *et al.*, 1996) and between 3-7% at different high-risk groups in Eritrea (Omer *et al.,* 2002).

Veterinarians who handle infected cattle, aborted fetuses and placentas without adequate protection who are at risk of the infection (Seifert, 1996). This is in consistent with Memish, (2001) who reported 22.5% of human brucellosis cases in Saudi Arabia, were identified from the risk groups that had direct contacts with domestic animals. Conversely it was perhaps surprising that in the current study, none of the risk groups except the herdsmen tested positive for *Brucella* antibodies. However, this findings concured with Kubuafor *et al.,* (2000) in Ghana and Gebretsadik (2005) in Tigray. In the present study, no female was positive to *Brucella* antibody. This could be due to small size sampled. On the other hand Cooper (1991) had reported comparable findings in Saudi Arabia where the proportion was 5.4% and 6.34% per 1000 per year in females and males respectively with no significant differences. Similarly Mussie (2005) found equally susceptibles to brucellosis with seroprevalences of 4.17% and 3.39% in females and males respectively in Bahridar.

Brucellosis in humans is acquired from infected animals through direct contact or indirectly by ingestion of animal products (Acha and Szyfers, 2001). At the present study an association of *Brucella* seropositive with removal fetal membranes was observed. That is in agreement with Al Sekait (1999) that conducted a sero-survey for brucellosis in Saudi Arabia of who lived in rural and urban areas. A proportion of 26.6% of brucellosis cases were found in rural people who were exposed to infected animal and 9.5% in urban people. Therefore, under the existing high needs of animal products in the country there exist no justification to ignore the role of zoonotic diseases and choose a handicapped approach of promoting care handling and hygienic use of animal and animal products.

5. CONCLUSIONS AND RECOMMENDATIONS

Results of the present study revealed that bovine brucellosis is widely distributed in the western zone of Tigray. The presence of positive seroreactors were in mutually exclusive with the strategy of the regional government of providing cattle to farmers. Therefore, the findings of positive serological reactors does not only suggest the occurrence of the disease in cattle population of the study area, but also indicates the presence of foundation (foci) of infection that could serve as sources of infection for the spread of the disease into unaffected animals around and elsewhere in the districts. The production systems jointly with host risk factors as well as husbandry and management systems were found important risk factors associated with *Brucella* seroreactors. An association also was observed between aborted cows and seropositivity of the infection. Moreover, the study indicated that the disease existed in human beings who were among the high risk groups. This emphasizes impact of brucellosis in public health and the need to control and prevent brucellosis in the study area.

Based on the current findings the following recommendations are forwarded:

- Extension service system should be improved to combat the disease transmission to livestock and owners, simultaneously with regional government
- Screening test should be carried out before distribution of *Barka* cattle breeds from the study area to other districts
- Strict control of illegal animal movement to different districts, regions, and neighboring countries should effectively and legally imposed
- Health check up of the distributed cattle with reference to the disease and other causes of infertility ought to be accomplished, with the aims of isolation and hygienic care
- Proper hygienic practices and good husbandry management system (specially watering point) should be exercised
- Isolation of aborted animals, culling of cattle having infertility problem from the herd, and proper disposal of aborted fetuses and fetal membranes
- In the extensive production system, test and slaughter of positive animals with compensations payment to the farmers could be applied
- Vaccination needs to be carried out in the high endemic areas for seroreactors

- In order to realize the exact status of the disease at the study area in *Barka* cattle breeds with their risk factors and other cofounders detailed research need to be designed and conducted
- A teamwork between veterinary and human health personnel is extreme important to create awareness via educational campaigns among human risk groups about this zoonosis importance of the disease through easy educational channels

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7. ANNEXES

Annex 1: Questionnaire for individual owners

General information

Date________Region__________Zone_________District__________PA_______Village___

Name of respondent _________Age_________Sex_________Qualification ________ Family size_______Herd size________

Herd inventory

-Breeding females

-Dry (non pregnant)

-Pregnant

-Lactating

-Non-breeding females

- Heifer

- Un weaned female

-Breeding males

Un weaned males

-Non-breeding males

-Castrated males

Animal acquisition (born, purchased, gift) and disposed (sold, dead, gift)

Animal type	Animal entered the herd				Animal left the herd				Remarks
	n	Reason	Source	Year	n	Reason	Source	Year	
Breeding Female									
Calf (male)									
Calf (female)									
Young (male)									
Young (female)									
Adult (male)									
Adult (female)									

n=Number of animals

Management information

1. What types of livestock species are you keeping in the area? (cattle, sheep, goats, equine, camels, others)
2. Do you move your animals to other areas in search of feed and /or water ? (yes, no)
3. What is your grazing system? (communal, individual, both)
4. What is the water source of your animals? (river, spring, pond, well, borehole, other)
5. What type of house do you use for your animals? (only fenced, fenced with shed, other)
6. What type of mating do you use? (artificial insemination, natural, both)
7. Do you buy or sell animals from or/to the neighboring countries ?

Disease information

-What were the most important cattle diseases encountered in your herd during the previous year?

-Local name______ Major symptoms_________Scientific name_________

-Do you know any disease that causes abortion in cattle, sheep and goats? (Yes, no). If yes, what are they? Local name______ Scientific name_________

-Do you know a disease characterized by abortion at the last pregnancy and usually followed by retain fetal membrane? (yes/no). If yes, what is the local name of the disease?

-Have you observed abortion or stillbirths in your herd ? (yes/no)

-Do separate cows, sheep and goats during parturition ? (yes/no)

-What do you the aborted fetus and/or after birth ? (bury, throw, giving to dogs, doing nothing, others)

-Have you observed swelling on knee or testicles in your animals ?(yes/no)

Annex 2: Questionnaire format for risk groups

What do you do the milk?

-Home consumption

- Cash income
- Both
- Do you drink boiled or raw milk?
- What other milk products do you use?

Do you slaughter animals at home? If yes, for what reason?

- Home consumption
- Group share
- Ceremony
- Emergency slaughter

How do you consume meat?

- Cooked
- Raw
- Other treatment

General information

Wereda	sex	Age	Raw milk con.	Raw meat con.	Removal of placenta	Fever	Head ach	Night sweat	Back ach	Chills	Fatigue	Anorexia	Weakness	Joint pain

Activities and labor divisions

Activities	Young		Adult	
	Male	Female	Male	Female
Herding				
Watering				
Milking				
Delivery assistance				
Mating assistance				

Annex 3: Questionnaire format for serum sampling

Date____________

Region__________

Zone____________

District__________

PA_____________

Village (herd)_______

Owner name________

Risk factors					Reproductive performance and disease symptoms					
Sex	Breed	Age	Production system	Herd size	Calving interval		No.of caving	No.of abortion	No.of still births	Presence of hygroma
					<15 months	>=15 months				

n=number of animals, No=Number

Information obtained on the distributed *Barka* cattle breeds elsewhere the study area

Districts	Number of cattle entered	Year	No of cattle at Present (2000)	Reason for deduction
Samre	32	1995	19	Prolonged calving
Kola Temben	26	1996	15	Prolonged calving
Adigudom	22	1995	13	Prolonged calving
Atsebi	32	1996	17	Prolonged calving
Alamata	27	1996	17	Prolonged calving
Wukro	29	1995	15	Prolonged calving

Source: Office of agriculture and rural development of the respective districts

Annex 4: Serological test

Materials and reagents of Rose Bengal Plate Test

Glass slide, micropipette, micropipette tips, mixing applicator, magnifying glass, RBPT *Brucella* antigen, positive and negative control sera (from Mekelle regional laboratory)

Materials and Equipment of Complement Fixation Test

.

Equipment

Micro titer plates (96 U shape well), sealig-tape, multi channel and mono channel micropipettes, pipette tips, universal bottles, stirrers, weighing cylinder balance, thermometers, PH indicator, incubator, water bath, refrigerator, centrifugator

Buffers, preservatives and reagents

Diluents: veronal with calcium and magnesium (VCM) at PH 7.2 that was prepared in 5 fold concentration and stored at +4.

Preservatives

Alsever's solution (for preservation of sheep red blood cells)

Richardson solutions (for preservation of complement)

Reagents (Biological reagents)

Test sera (serum collected from study area)

Standard *B.abortus* antigen, positive and negative control sera were obtained from the BgVV, Berlin Germany

Complement (Guinea pig, animal stock NVI)

Sheep red blood cells (animal stock NVI)

Amboceptor (anti-sheep-haemolysin)

Preparation of reagents

i. Preparation of SRBC for hemolytic system

10ml of SRBC in Alsever's solution were centrifuged at 2500 rpm for 5 minutes. The supernatant was discarded and replaced by veronal buffer diluents. The SRBC were resuspended in the diluent and centrifuged again. This procedure was repeated 4 times. Before discarding the supernatant after the last washing, the packed cells volume was measured. The volume of the packed cells volume was read by placing an identical tube next to the blood containing tube and filled up to the level of the blood by a measured amount of water. Finally a 2% suspension of SRBC was prepared.

ii. Amboceptor titration

- Two rows of 5 test tubes each were arranged on rack
- In two other test tube, 1:500 and 1:750 prediluted were made
- 1ml of 1:500 prediluted amboceptor was transferred to the first test tube of row one and 1ml of 1:750 prediluted amboceptor was transferred to the first test tube of row two
- 0.5ml of VBD was added to each of the test tubes of both row
- Amboceptor was then diluted serially from tube 1 to tube 5 with an amount of 0.5ml in both rows. Thus the dilution ran from 1:500 to 1:800 and 1:750 to 1:12000 in row 1 and row 2, respectively
- To each tube of the two rows, 1ml of VBD was added
- Following, 0.5ml of 2%SRBC was added to each test tubes of the two rows and were shacked well
- The tube were left on the table for 10 minutes
- 1ml of complement at working dilution was added and incubated at 37 ^{0}c for 30 minutes
- The last tube showing complete hemolysis, minimum hemolytic dose (MHD) was read.

iii. Evaluation of complement

- Freeze dried complement was reconstituted according to its instructions
- A 1:100 complement dilution was prepared
- Complement was added into 9 wells increasing by 5micolit every time, starting with 10microlit
- Diluent was added into the 9 wells in decreasing amounts by 5microlit, starting with 40microlit
- 25microlit of a diluent was added into the wells with Cornwall syringe
- The plate was placed in water bath at 37 ^{0}c for 1 hour
- 25microlit 2% SRBC was added to all walls
- 25microlit amboceptor at working dilution (1:1000) was added to all wells
- Components were mixed by shaking and incubated again in water bath at 37 ^{0}c for 30 minutes

The test was read by recording minimum hemolytic dose of complement (MHD), which was represeented by the first well showing complete hemolysis. The next well contains the full hemolytic dose (FHD). The working dilution of complement was then computed: complement dilution=2FHD/initial dilution of complement.

iv. Antigen titration

Micro titer plate I

- 25μl of VBD was first placed to every wells of a micro titer plate
- 25μl of a prediluted antigen was added to all wells of row A
- By serial (2 fold) dilution 25μl of antigen was transferred from row A to B and from row B to C until row G by multichannel pipette and 25μl mixture was discarded from row G

Micro titer plate II

- 50μl VBD was added in all wells
- 50μl of prediluted inactivated positive control serum was added to all wells of column one

- 50μl was serially transferred by 2 fold dilution, from column one to two, and again from column two to three, until column 11 from where 25μl was discarded (column 12 had only VBD)

Mix plate I and II

- 25μl was transferred from plate II to plate I
- 25μl of complement at working dilution was added to all wells of plate I
- Plate I was incubated at 37 ^{0}c for 30 minutes (hot fixation)
- The following 25μl of equal volumes of 2% SRBC and amboceptor (working dilution) pre-mixed were added to all wells
- The plates were covered with sealing tape and placed in an incubator (37 ^{0}c) for 30 minutes (warm fixation)

Interpretation: the last wells with 50% sedimentation was read and recorded. This was regarded as the right corner value. In this case, the corner value was 1:25 dilution and was used through out the test. The 50% sedimentation was taken as one unit and the working dilution of the antigen was two units

8. CURRICULUM VITAE

Personal data

Name: Mekonnen Haileselassie Wereta

Date of Birth: 27 April 1981

Place of Birth: Saesie Tsaeda Emba wereda, Eastern Zone of Tigray, Ethiopia

Sex: Male

Marital status: Single

Nationality: Ethiopian

Educational background

2007-2008: Addis Ababa University, Faculty of Veterinary Medicine, Ethiopia, MVSc follow: in Tropical Veterinary Public health

1998-2004: Addis Ababa University, Faculty of Veterinary Medicine, Ethiopia, Graduated with degree: Doctor of Veterinary Medicine (DVM)

1995-98: Grade nine to twelve Agazi Comp. Sec. School, Adigrat, Tigray, Ethiopia

1987-95: Grade one to eight Mymegelta Elementary School Eastern Zone of Tigray, Ethiopia

Work experience

2004/2005: Field veterinarian in western zone of Tigray, wereda Kafta Humera

2005/2006: Team leader of animal production development and veterinary science department and Live animal export inspection and certification in western zone of Tigray, wereda Kafta Humera

Extra curricular activities

2001-2002: Activities on Area Development Program under the World Vision, Ethiopia.

2003: Collection and Identification of Medicinal Plants in Tigray, under the Conservation and Sustainable use of Medicinal Plants Project (CSMPP).

Special skills

Computer skill in MS DOS, MS Window, Ms Excel, MS Access

Languages

Tigrigna: speaking and writing
Amharic: speaking and writing
English: speaking and writing

Publications

2008: Master of Veterinary Science

Title: Seroprevalence study of bovine brucellosis and its public health significance in western zone of Tigray, Ethiopia

2004: Doctor of Veterinary Medicine Thesis

Title: Survey and Preliminary screening of selected medicinal plants for treatment of bovine mastitis and skin diseases in Tigray, Northern Ethiopia

. 2003: A Seminar on Concepts and Problems of Livestock Development.

Title: Effect of *Phytolacca dodecandra* (Endod) on causative agents and vectors of some animal diseases

References

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9. SIGNED DECLARATION SHEET

I, the undersigned, declare that the thesis is my original work and has not been presented for a degree in any University and that all sources of material used for the thesis have been duly acknowledged.

Name: Mekonnen Haileselassie

Signature: ____________________

Date of submission: 25-06-08

This thesis has been submitted for examination with our approval as University advisors:

Dr. Moses Kyule (BVM, MSc, MVPM, PhD) (Associate Professor) ____________________, 25.06.0

Dr. Kelay Belihu (DVM, PhD) (Assistant Professor), ____________________

Dr. Shewit Kalayou (DVM, MSc, Assistant Professor) ____________________